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修士論文

Is green light ineffective for
the growth of plug seedlings?

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General Introduction

Historical studies on green light and its effectiveness on plant growth and development

Light is one of the most important factors playing key roles on the plant growth and development. Photosynthesis and photomorphogenesis are the two distinct ways in which terrestrial plants use radiant energy. For the photosynthesis, it has been concluded that photons in only wavelength region between 400 and 700 nm can be used as energy source for the photosynthetic mechanism. By the investigations on leaves, photons in wavelength regions of blue (400-500 nm) and red (600-700 nm) could induce the higher CO₂ assimilation rate compared with the green light (500-600 nm) (McCree, 1972). The lower absorption of green light by the leaves calculated by the higher transmittance of the single leaves, seem to be the conclusive reason, since absorption spectra of isolated photosynthetic pigments and leaves matched closely with the photosynthetic action spectrum of higher plants when CO₂ assimilation was determined with equal energies of visible wavelength (Noggle and Fritz, 1976; McCree, 1971; 1972). Because the green light was assumed to be less effective for the photosynthesis and less absorbed by the leaves, most of researches in the next generation assumed that the green light is unnecessary and also sometime ineffective compared with other wavelength regions of light. However, it should be noted that the studies and measurements of the absorptance of leaves by McCree (1972) suggested that neither the mean action spectrum nor the mean quantum yield could be flat throughout the visible region. Therefore neither the irradiance nor the flux of absorbed radiation will be a perfect measurement of the real

photosynthetically active radiation, while the irradiance is overestimating more than the photon flux. Also, Duysen (1952, 1956, 1957), who is the first to apply the measurement of changes of absorption induced by illumination to the study of photosynthesis, suggested that even a properly measured absorption spectrum may not be identical to that of the same pigments in solution, if the pigments are contained in particles that have a non-negligible absorption (flattening effects).

Amesz (1996) reviewed that the absorption spectra of rather poor quality are being published occasionally even today, and this is mainly due to the fact that the commercial apparatus normally used are not designed for scattering materials. If the light scattering is not properly corrected, not only causes an upward shift and distortion of the absorption spectrum, but it may also decrease the amplitude of absorption bands (Amesz et al., 1961; Latimer and Eubanks, 1962) and the additional distortion may occur due to the selective scattering near the absorption bands (Latimer, 1959). Therefore, many methods have been suggested for the higher accuracy in measurement such as using the sphere-shape chamber connected to the photo-sensor required for collecting all the radiation reflected from the surface of leaves, and the chamber needs the strong direct beam of light to minimize the scattering and spreading of radiation. In contrast, for the transmission, it has been suggested that the errors of measurement can be minimized by collecting the transmitted light over a relatively large angle (Amesz, 1996). Nevertheless, there were other difficulties in choosing the samples, and the high variation due to many factors such as the containing materials, age, physiological structure, growth status of the photosynthetic materials or leaves. The size of error depends on the spectral quality of the

light source (McCree, 1972). Also the investigation under various percent green light has not been determined.

Therefore, the above statements suggested that the clear explanation for effectiveness of light in wavelength region of green (500-600 nm) is still necessary. Thus in parts of this study also includes attempts to measure transmitted light by using light source providing relatively narrow wavelength region of light.

Advantages of artificial light in intensive study of plant response

Artificial light provides more controllability compared with the natural light. Light environment including intensity, duration, quantity and quality plays important roles on plant growth and development. To determine those responses, the uncontrollability of solar radiation limits its application for intensive studies of plant responses. Therefore, the artificial light is considered advantage for the intensive study of plant responses.

Increasing light quantity is a simple way to promote the growth of plants because it can theoretically increase the photosynthesis of plants. However, the over increased light intensity can cause stress to the plants and also may be resulted in the shut down of photosynthesis, i.e., photoinhibition (Styer and Koranski, 1997). Also, it should be noted that either increase of light intensity, duration, or quantity resulted in the higher electrical energy consumption and production cost in plant production. Therefore, optimizing growth with the increased light intensity has been emphasized in several researches.

By using various kinds of lamps as the light sources, quality of light has been proved to be effective on controlling plant morphogenesis. Even

though, the broad spectra of light provided by those light sources limited the efficiency of investigations. Thus, interference filters have been applied to eliminate the excessive wavelength regions. Red and blue lights are known to be effective for the photosynthesis and growth of individual plants mainly because the leaves have high absorption of light in blue and red regions. The peaks of leaf absorption spectra measured in many plant species are at red region between 655 and 665 nm and blue region between 445 and 455 nm (McCree, 1972). In addition, Shull (1929) reported that maximum reflectance of broad leaves is at green region between 540 and 560 nm.

Also, the different effectiveness of light in different wavelength regions on photosynthesis indicated the influences of the light quality on photosynthesis of plants. Thus, the manipulation of light quality should be considered one effective way to promote the growth and development of plants, but does not increase the energy consumption, however, it will increase the capital cost for changing the lighting system, which is the same amount as when renewing the light source.

During the last decades, light emitting diodes (LEDs) have been developed for provide relatively narrow wavelength regions of light (Williams and Hall, 1978; etc.). LED has been broadly used and its most popular applications are as indicators or optoelectronic switches for electrical appliances. Several recent advances in the LED technology improved the practical use of LEDs for applications in the fields that require a high photon flux, such as for the lighting system for plant productions. In addition, several types of LEDs were also developed for providing the specific light compositions and they can be the useful light sources for the study of light responses on the plants (Bula et al., 1991; Bula et al., 1994).

Importance of plug seedling use

Plug is known as a containerized transplant which has many advantages over bare-root seedlings or conventional seedlings. There are many advantages of plug seedlings such as:

- Less time and labor to transplant
- More uniform growth after transplanting
- Increased production per area
- Can be held or delayed before transplanting
- Less chance for disease to spread

Therefore, plug seedlings have been introduced and used especially in the large-scale plant productions. However, the commercial use of plug seedlings has been limited because of some disadvantages such as:

- Require skillful labor to produce high quality plug seedlings
- Longer time to produce plug seedlings compared with seedling bed
- High initial cost for the production of plug seedlings, greenhouse

The quality of transplants is one of the most important factors to be critically emphasized in plant productions. Therefore the plug seedlings are suitable for the growers who recognized benefit of using high quality transplants (e.g., Styer and Koranski, 1997).

For plug seedlings, or high planting density crops, the shading responses that caused by the near-by seedlings and the canopy of leaves greatly influence the growth and habits of those seedlings or crops (e.g., Styer and Koranski, 1997; Papadopoulos and Pararajasingham, 1997). The crop photosynthesis is not only dependent on the distribution of the PPF among the layers, but also on the total amount of photosynthetic photons (PP) absorbed by the canopy (Duncan, 1971; Norman, 1980). Manipulation of

planting density (or planting space) to optimize the PPF interception is one possible strategy for increasing yields of tomato (Papadopoulos and Pararajasingham, 1997), soy bean (Shibles and Weber, 1965), cereals (Gallagher and Biscoe, 1978) and orchard crops (Jackson, 1980). The radiation can be absorbed, reflected by and transmitted through the leaves depending on their wavelength regions, therefore, the spectral distribution of the solar radiation (both diffuse sky and direct beam) also changes inside the canopies. It was found that the canopy structure, including the distribution of gaps and surface orientation inside the canopy, the scattering processes in the canopy and also phytoelements inside the canopies directly affect changes in the spectral irradiance with depth and varying orientation in the canopy (Coombe, 1957; Federer and Tanner, 1966; Robertson, 1966; Vezina and Boulter, 1966; Kinerson, 1973; Goodfellow and Barkham, 1974; Holmes and Smith, 1977; Tasker and Smith, 1977; Turnbull and Yates, 1993; Grant, 1997). Therefore, consideration of only light intensity at the top of canopy level is not enough anymore. The further investigation of seedling growth in high planting density crops is necessary and should be determined.

Objective of the present study

The present study was conducted to examine the effects of green light on the growth of plug seedlings. Firstly, the growth of tomato plug seedlings under three various percentages of green PF at a given PPF level was compared using LED light source. Secondly, the transmissions of green, blue, and red light through the tomato leaves were investigated. Thirdly, the fluorescence emitted from the tomato leaves was observed and its relation to the transmittance was compared under various light qualities.

Chapter 1

Effects of percent green PF on the growth of tomato plug seedlings

1.1 Introduction

Blue and red lights are effective in the photosynthesis of leaves, and also play many important roles on plant growth and development. On the other hand, only a few reports characterized the effects of green light on plant responses since the green light was found to be less effective in photosynthesis and less absorptance of the individual leaves compared with blue and red light. However, the growth of seedlings under various percent green photon flux (percent green PF) at a given photosynthetic photon flux (PPF) has not been investigated, especially for growth of canopy seedlings.

During the last decades, LEDs have been developed for providing relatively narrow wavelength regions of light. By using the combinations of LEDs, it becomes more practical to make the various specific light qualities (e.g., William and Hall, 1978). Blue, red and far-red LEDs have been successfully used in the investigation on plant responses as affected by the light in the specific wavelength regions. With the advanced technology in LED, the green LED has been developed, but its applications on the studies of plant responses are rarely found.

The present experiment was conducted to examine the effects of percent green PF on the growth of tomato plug seedlings by using green, blue, and red LEDs as light sources.

1.2 Materials and methods

1.2.1 Lighting system

Green (1188 lamps, peak at 535 nm, serial No.: NSPG300A; Nichia Chemical Co. Ltd., Japan), blue (1188 lamps, peak at 475 nm, serial No.: NSPB300A; Nichia Chemical Co. Ltd., Japan) and red (2376 lamps, peak at 650 nm, serial No.: HLMP8103; Hewlett Packard Co. Ltd., Japan) LEDs were systematically arranged in an LED panel. The LED panel was set over a specially designed box (i.e. LED box), used for providing green, blue and red light. Light intensity output of green, blue and red LEDs can be adjusted independently by using the electric current controller. For the study of various percent green light using only one LED box, a panel of fluorescent lamps (FLR40S EX-N/M-X-36; National Co. Ltd., Japan) set above a separate box (i.e. fluorescent lamp box) was used for the references repeated at each time of experiments conducted. Spectral photon flux of the green, blue, red LEDs and fluorescent lamp were measured by using a portable spectroradiometer (LI-1800; LI-COR Inc., USA). The spectral distributions in relative spectral photon flux are shown in Fig. 1. Characteristic and maximum output of each color of LEDs were used in consideration of treatments. The photosynthetic photon flux (PPF) and blue (termed B; 400-500 nm) photon flux at the top of canopy level were needed to be the same for all the treatments. The green (termed G; 500-600 nm) PF varied for three percent green PF of 24%, 12% and 1% treatments and red (termed R; 600-700 nm) also varied to maintain the same PPF for all the treatments. Photoperiod was 16 h d⁻¹ for all the treatments. The photon flux component in photon flux and in percentage of PPF and the

spectral distributions of each treatment are shown in Table 1, Table 2 and Fig. 2, respectively. It should be noted that the far-red (termed FR; 700-800 nm) PF was greatest in fluorescent lamps compared with the LED.

1.2.2 Plant material

Tomato (*Lycopersicon esculentum* Mill., cv. Momotaro; Takii seed Co. Ltd., Japan) plug seedlings were grown in cells (H 49 mm×W 29 mm×L 31 mm; 25 ml in volume) of plug tray (2x4 cells per tray; 64 mm×124 mm). The cells were filled with commercial soil mixture (Yanmar Co. Ltd., Japan). Seeds were sown into the soil mixture and then the plug trays were covered with white paper for two days. After germinating under the dark, seedlings continued growing under the treatment of light for another one week. Then, the uniform healthy seedlings were selected to make uniform canopies of seedlings. The cells were separated from each other and rearranged into another connected plug tray to prevent from the stress that might occur during the selection with the maintained planting density after selecting. Then, the seedlings continued growing at the same environmental conditions for the remaining of experimental period.

1.2.3 Environmental conditions and controls

Both LED box and fluorescent lamp box were placed inside the growth room with controlled environmental conditions of 26°C/22°C air temperature (photo-/dark period), and 65% relative humidity. Data of temperature and relative humidity inside the growth room and inside the both boxes were collected by using a data logger (21X; Campbell Co. Ltd., USA). Heaters and fans were set up inside the two boxes and they were controlled by using signal output from the programmed data logger and a personal computer. Relative humidity inside the growth room was controlled by using a humidifier connected to the relative humidity controller (Humid-eye; Saginomiya seisakusho Inc., Japan).

In order to give the uniform environmental conditions inside the boxes for all the seedlings, the position of plug trays were rearranged everyday. Jacks were used for maintaining the distance between the light source and seedlings when height of seedlings increased.

1.2.4 Growth measurement

On day 15 after sowing, the growth of the seedlings was determined by measuring total fresh weight, leaf fresh weight, cotyledon fresh weight, petiole fresh weight, root fresh weight, and stem length. Shoot fresh weight was calculated by the sum of leaf, cotyledon, petiole and stem fresh weight. Dry weight was measured after drying the samples in a hot air oven at 60°C for one week.

1.2.5 Leaf area measurement

Images of leaf blades from each tomato seedlings were photocopied immediately after excising. By using an image scanner (GT-8500; Seiko Epson Corp., Japan), the images of leaf blades were converted to computer files. Then, the area of the images was measured and calculated by using a measuring program (Fujitsu engineering Co. Ltd., Japan).

1.2.6 Experimental design and statistical analysis

Because there was only one LED box for comparing three different percent green PF, three experiments were separately conducted. The similar experiments under fluorescent lamps were repeated at the same time.

Average value from eight seedlings was used as one replication. There were eight replications in each treatment. Statistical analysis by t-test at $\alpha=0.05$ was used for comparing the growth of seedlings in each treatment under LED to those of the seedlings under fluorescent lamp conducted during the same time.

As the cooling system of the growth room was not functioning optimally during the experiments, temperature fluctuations may have influenced the experimental data. However, the average temperatures were the same among the treatment, and the same pairs of treatments conducted together would have the same environmental conditions.

The three parts of this study were done separately, so the differences of environmental conditions occurred during the experiments might affect the growth of plug seedlings. Therefore, the absolute values of growth may not be able to use in comparisons among treatments. There were two methods to compare the growth of seedlings under G24%, G12% and G1%.

1) Relative growth (R), calculated using the following equation

$$R = G / F$$

where G is growth of seedlings under G24%, G12% and G1%, while, F is growth of seedlings under fluorescent light in the respective treatments.

2) Specific different growth (S), or differences between the growth of seedlings under LEDs and those under fluorescent lamps, calculated using the following equation:

$$S = (G - F) / F$$

ANOVA was used in comparison among the growth indexes of G24%, G12% and G1% seedlings. Then the least significant difference test (LSD) at $p \leq 0.05$ was used for comparing between each treatment. Also, It should be noted that the growth under G24%, G12% and G1% could not be compared with the growth under fluorescent light of the present study because the treatment is not continuously additive model which will result in an apparent heterogeneity of error due to false assumptions when no transformation is made before analysis. However if comparison will be made with the growth under fluorescent light (G44%), the G24% and G1%, the treatment can be considered the additive model treatments. Therefore another comparison method was used for comparing the growth of seedlings under fluorescent light (G44%), G24%, and G1%.

3) Absolute growth values, since there were no significant differences between the repeated treatments under fluorescent light conducted together with G24% and G1%, therefore, the absolute growth values of G44%, G24% and G1% were compared by using ANOVA. Also the comparisons between treatments were made using LSD test at $p \leq 0.05$.

1.3 Results and discussions

1.3.1 Growth characteristics

The absolute growth of tomato plug seedlings on day 15 after sowing is shown in Table 3. The relative growth and specific growth of seedlings are shown in Table 4 and Table 5, respectively.

For the fresh weight, there was no significant difference between the total fresh weight of G24% seedlings and that of the seedlings under fluorescent light, while the total fresh weight of G12% and G1% seedlings were significantly greater than those of seedlings under fluorescent light. In other words, total fresh weight of the seedlings increased with decreasing green PF.

For the dry weight, only the total dry weight of G1% seedlings was greater compared with those of seedlings under fluorescent light. However, there was also no significant difference in the relative total dry weight and specific total dry weight among all the treatments. The slightly increased dry weight might be attributed to the leaf expansion as affected by the red PF, data as shown the next section. However, Papadopoulos and Ormrod (1988) reported that the net photosynthesis of the upper part was 1.43 times higher than that of the lower parts at 23 cm spacing, while they were nearly the same at 45 cm spacing. Therefore, since the plug seedlings were grown in a high planting density, the interception of light within the canopy could be also considered a factor influenced the increasing of dry weight.

However, when comparing the absolute total dry weight of tomato plug seedlings under G1%, G24% and fluorescent light, there was also no significant difference among the total dry weight of those seedlings.

1.3.2 Expansion of cotyledons and leaves

Area, relative area, and specific area of cotyledons and leaves are shown in Table 3, Table 4, and Table 5, respectively. The results showed that only cotyledons and leaves of G1% seedlings were larger than those of seedlings under respective fluorescent light and also were the largest among treatments of 3 various percent green PF.

Fukuda et al. (1996) has previously reported on tomato plants, grown by hydroponics using color lamps as the light sources. Red (600-700 nm) and yellow (550-600 nm) lights affected the expansion of leaves, while green (500-550 nm) and blue (400-500 nm) light resulted in reducing the leaf size.

From the results of the present study, however, it can be concluded that the green light (500-600 nm) suppressed the expansion of tomato seedling cotyledons and leaves because even the G1% containing a given blue PF, the enlargement of leaves could be observed. In addition, the response was the same in cotyledons. Thus, the expansion of cotyledons and leaves may attribute to the increasing dry weight and fresh weight of G1% seedlings.

1.3.3 Elongation of stem

The relative stem length of G24% and G12% seedlings were shorter compared with that of G1% seedlings, respectively. In other words, the stem length of seedlings was significantly decreased with the increasing percent green PF. However, there was no significant difference between stem length of G1% seedlings and that of seedlings under fluorescent light.

In addition, since the decreasing height, as affected by increasing percent green PF, of seedlings grown under fluorescent light could not be clearly shown, it might be explained by the effects of FR light that influenced the response of seedlings against green light. However, the ratio of shoot dry weight to stem length of seedlings grown under LED light was about 20% greater than that of seedlings grown under the fluorescent light, while, there was no significant difference among seedlings grown under LED light. These results also confirmed that the stem elongation of seedlings under fluorescent light was affected by the far-red PF, which was also the highest in the fluorescent light. (Downs et al., 1957; Decoteau and Friend, 1990; Miyashita, 1994; Kirdmanee et al., 1996). Moreover, the results suggested that the effects of light quality on both hypocotyl and epicotyl elongation were the similar since there were no significant difference in the ratios of epicotyl/ hypocotyl length of those seedlings.

1.4 Conclusions

Total fresh weight of tomato plug seedlings decreased with increasing percent green PF of the light source. However, the changed percent green PF and percent red PF with maintaining PPF level did not affect the total dry weight of plug seedlings. In other words, green light is as effective as red light on growth of plug seedlings in term of dry weight.

The various percent green PF did not directly affect the growth of plug seedlings without the process of photomorphogenesis. The expansion of cotyledons and leaves could increase dry weight of seedlings, but the difference was not significant. This might be caused by the overlapping leaves, which is difficult for light to intercept the seedlings inside the canopy.

The light quality shows the effects on leaf and cotyledon area, stem height and compactness of plug seedlings. Therefore, it means that the quality of seedlings could be improved by the mean of light quality manipulation.

The plug seedling growth has close relationship with the seedling height, since the leaf canopy get closer to the light source. The vertical level adjustment should be recommended for the precise study of light responses at a given light intensity on the canopy level.

Chapter 2

Transmission of blue, green, and red through tomato leaves

2.1 Introduction

A canopy structure mainly influences its own microenvironment and the light interception by surrounded leaves (Nobel et al., 1993). The growth of plug seedlings mainly depends on the intensity of light incident upon the leaves, however, unexpected appearances of plug seedlings like necrotic leaves, uneven growth etc. may occur under the over increase of light intensity (Styer and Koranski, 1997). That is because the upper leaves received almost of the light incident upon them, and thus the seedlings inside the canopy will be elongated due to the shading responses. Attempts to increase planting density has been continuously studied in order to increase the utilization of space and to decrease the production cost per plant. However, dramatic decrease in the growth of plug seedlings with the increasing planting density has not been overcome. Thus, the ideal light which can be evenly absorbed by leaves in vertical orientation is necessary.

The objective of this experiment was to investigate the quality and intensity of light under the layers of tomato leaves under various percent green photon flux and under the green, blue or red light of LEDs. Differences in characteristics of the light under the leaves of seedlings and that of mature tomato plants from a field were also studied.

2.2 Materials and methods

2.2.1 Plant materials

Intact unfolded leaves were obtained from tomato plug seedlings grown in the culture room at $230 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF and 16 h d^{-1} photoperiod provided by white fluorescent lamps (FLR40S EX-N/M-X-36, National Co. Ltd., Japan), $26 \pm 2^\circ\text{C}$ / $22 \pm 2^\circ\text{C}$ air temperature (photo-/ dark period) and $70 \pm 10\%$ relative humidity. For the comparative study, intact leaves were also obtained from mature tomato plants cultivated in the field (Chiba, Japan; July-August 1997).

2.2.2 Measurement of light quality and intensity under the leaves

Blue (1188 lamps, peak at 475 nm; Nichea Chemical Co. Ltd., Japan), green (1188 lamps, peak at 535 nm; Nichea Chemical Co. Ltd., Japan) and red (2376 lamps, peak at 650 nm; Hewlett Packard Co. Ltd., Japan) LEDs or the fluorescent lamps (FLR40S EX-N/M-X-36; National Co. Ltd., Japan) were used as light sources. Spectral distributions of light under the intact leaves of tomatoes were investigated under the different qualities of light sources. A photo-sensor (remote-cosine receptor, LI 1800-1; LICOR Inc., USA) of the portable spectroradiometer (LI-1800; LICOR Inc., USA) was fixed on the stand. Then, the leaf samples were placed over the photo-sensor, and fixed by white paper with a hole in the middle. From the preliminary observations, the continuous measurements did not cause any significant differences, but changes in the measuring points caused significant differences between each time of measurements. Thus, the same samples were used for the measurements under every light quality. The

spectral photon flux was investigated in wavelength region between 330 and 1100 nm. It took about one minute to complete one cycle of measurement by the spectroradiometer and another one minute to prepare the proper light quality by manually adjusting the electric current output of the LEDs. The measurements were conducted at $23\pm1^{\circ}\text{C}$ air temperature, and 60% relative humidity. The transmittance, an index to determine transmission, was calculated from the ratio of photon flux of light transmitted through the layer of leaf samples to the photon flux reading without any leaf samples.

2.2.3 Experimental design

There were two factors, light quality and number of leaves, in the experiment. The factor of light quality included three complementary colors, blue, green and red light, and also their combinations, as used in Chapter 1. The number of leaves was one (single) and two (double) leaves.

2.3 Results and discussions

2.3.1 Transmittance and the quality of incident light

As shown in Fig. 3, Fig. 4 and Table 6, the transmittance of green light was larger compared with those of red light, respectively. Therefore it could be assumed that the light with more green PF could effectively penetrate more layers of tomato leaves. In other words, the PPF detected inside the canopy should be higher under the light of higher green PF.

Also, the transmittance of light with higher green ratio was greater than that of light with lower green ratio (Table 7). Under the second layer of leaves, however, the light intensities were nearly the same among those light qualities. Most of the PF might be absorbed at the second layer of leaves before it can be detected by the photo-sensor. Thus, it can be concluded that the light transmitted through the first layer of leaves can be absorbed by the second layer of leaves, and the green light is considered evenly absorbed by the layers of leaves.

However, it should be noted that the leaves in nature are not perfectly overlapped, therefore not only the transmitted light but also reflected light and scattered light through the canopies should be get into consideration.

2.3.2 Changes in light quality

From the spectral distributions of light under the tomato leaves, not only the intensity but also the quality of light reached to the lower layers of leaves were also changed, as shown in Fig. 5, Fig. 6, Fig. 7 and Fig. 8. Within the PPF region, green ratio of light was increased after transmitting through the first layer of leaves, while blue and red ratios were decreased. The increased percent green ratio of transmitted light might be attributed to the higher transmittance of wavelength regions of green than that of other wavelength regions.

When considering the PPF with FR region, the ratio of PPF:FRPF was decreased, and the PF ratios of B:FR, G:FR, and R:FR were also decreased with the increasing number of leaf layers that the light penetrated, while the decreasing rate was variable dependent upon the qualities of incident light. The phenomenon was the same as in the leaves of tomato mature plants. It might be attributed to the different transmitting rate of green, blue and red light through tomato leaves, as shown in Fig. 6, Fig. 8 and Table 6. The discussion about the increasing FR ratio will be discussed in Chapter 3.

2.3.3 Changes in patterns of spectral distributions

For the blue light using blue LED providing single peak within relatively narrow wavelength region, even though the shoulder of peak in wavelength region of 500-550 nm was observed after the light transmitted through the leaves of seedling or mature plants. In addition, the peak in wavelength region of 700-800 nm (termed FR peak) was detected, even there was no any peak in that wavelength region of the light incident upon the leaves, as shown in Fig. 9. For the green light, as shown in Fig. 10, regardless of the decreasing in light intensity, there was also the FR peak found with lower PF level compared with the blue light. For red LED, as in Fig. 11, the peak in wavelength region of 700-800 nm was also found with the minor peak in wavelength region of 680-700 nm. Furthermore, there was also the shifting of peak when using the leaves of mature tomato plants, but it was not found under the double layer of leaves.

The distribution of spectral photon flux of light transmitted through the leaves of tomato seedlings were different from those of light transmitted through the leaves of mature tomato plants. It might be dealing with the age of leaves, positions on the leaves, biochemical components inside the leaves, and culture conditions, etc., which may influence the optical characteristic of leaves (e.g. Krause and Weis, 1988).

The further discussion in this Chapter will be only on the shifting in wavelength region of blue. The presence of peaks in wavelength region of FR will be discussed in Chapter 3.

2.3.4 Changes in patterns of a peak in blue region

To discuss the changes in the peak pattern of blue LED, another measurement was conducted separately using sweetpotato leaves. The light from blue LED also included the PF in wavelength region of green, therefore, a blue filter (Hoya Co. Ltd., Japan) was used to eliminate PF in wavelength region of green. The results showed that the peak of the transmitted light was at the same position to the incident light for both the light from blue LED and the filtered blue light, as shown in Fig. 15. There were only decreasing in light intensity without any remarkable changes in pattern of the peak when using the filtered blue light. Thus it can be proved that the shifting or changing of the peak pattern in region of blue did not concern with any relation to the specific properties of photons in wavelength region of blue or shifting energy level of the photons. On the other hand, it could be explained by the differences in transmittance of light between the different wavelength regions of blue and green.

2.4 Conclusions

The quality and intensity of light below the canopy are depending upon the transmittance of leaves varied upon the wavelength regions. The higher transmittance of green light than those of blue and red light, respectively, affects the changes in quality of light under the layers of leaves. Furthermore, the increasing green ratio of the incident light can increase the transmittance of the tomato leaves. In other words, the green light could be evenly absorbed by the layers of leaves within the canopies.

The PF ratios of B:FR, G:FR, and R:FR were decreased under increasing number of leaf layers, but the decreasing rates were variable.

The tomato seedlings and plants showed the specific responses to light in limited range of wavelength regions. Therefore, it should be suggested to beware of choosing the light quality for intensive study on plant responses to the light. Laser, another kind of light source, has been developed for providing the monochromatic light in many specific wavelengths. It has also been used for applications in many fields of works such as engineering, science and technology, and agriculture. This kind of light source would be beneficial for the intensive studies on plants and their responses to light within the specific wavelengths.

Chapter 3

Chlorophyll fluorescence emitted from the tomato leaves

Introduction

From the Chapter 2, changes in the quality and intensity of light transmitted through the leaves could be observed by measuring light transmitted through the tomato leaves. In addition, a peak in wavelength region of 700-800 nm was found in the spectral distributions of the transmitted light while there was no any peak in the spectral distributions of the light source. It was considered the chlorophyll a fluorescence, which is well-known in several articles, since it is in wavelength region between 700 and 800 nm and it was emitted from the leaves exposed to the light. Kautsky and Hirsch (1934) were the first to report the pattern of fluorescence induction when dark-adapted leaves were exposed to light. Fluorescence emission spectra can also be detected from intact cell, isolated chloroplasts and thylakoid membranes. It was also found that the differences of the light qualities influenced the yield of that emitted light.

In this chapter, the light quality effects and fluorescence yield in relation to the transmission of light were discussed.

3.2 Materials and methods

3.2.1 Plant materials

Intact unfolded leaves were obtained from tomato plug seedlings grown in the culture room at $230 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF and 16 h d^{-1} photoperiod provided by white fluorescent lamps (FLR40S EX-N/M-X-36, National Co. Ltd., Japan), $26 \pm 2^\circ\text{C}$ / $22 \pm 2^\circ\text{C}$ air temperature (photo-/ dark period) and $70 \pm 10\%$ relative humidity. For the comparative study, intact leaves were also obtained from mature tomato plants cultivated in the field (Chiba, Japan; July-August 1997).

3.2.2 Measurement of light quality and intensity under the leaves

Blue (1188 lamps, peak at 475 nm; Nichea Chemical Co. Ltd., Japan), green (1188 lamps, peak at 535 nm; Nichea Chemical Co. Ltd., Japan) and red (2376 lamps, peak at 650 nm; Hewlett Packard Co. Ltd., Japan) LEDs or the fluorescent lamps (FLR40S EX-N/M-X-36; National Co. Ltd., Japan) were used as light sources. Spectral distributions of light under the intact leaves of tomatoes were investigated with the different qualities of light sources. A photo-sensor (remote-cosine receptor, LI 1800-1; LICOR Inc., USA) of the portable spectroradiometer (LI-1800; LICOR Inc., USA) was fixed on the stand under LED panel. Then, the leaf samples were placed over the photo-sensor, and fixed by white paper with a hole in the middle. From the preliminary observations, the continuous measurements did not cause any significant differences among the measurements, but changes in the measuring points caused significant differences at each time of measurement. Thus, the same samples were used for every light quality.

The spectral photon flux was investigated in wavelength region between 330 and 1100 nm. It took about one minute to complete one cycle of measurement by the spectroradiometer and another one minute to prepare the proper light quality by manually adjusting the electric current output of the LEDs. The measurements were conducted at $23\pm1^{\circ}\text{C}$ air temperature, and 60% relative humidity. The transmittance, an index to determine transmission, was calculated from the ratio of photon flux of light transmitted through the layer of leaf samples to the photon flux reading without any leaf samples. Fluorescence yield was calculated by the summation of photon flux in wavelength region of 700-800 nm divided by the PPF of the incident light.

3.2.3 Experimental design

There were two factors, light quality and number of leaves, in the experiment. The factor of light quality included three complementary colors, blue, green and red light, and also their combinations, as used in Chapter 1. The numbers of leaves were one (single) and two (double) leaves.

3.3 Results and discussions

3.3.1 Increases of photon flux in FR region

The peak, appeared in the spectral distributions of light transmitted through the leaves, could be detected in wavelength region of 700-800 nm, especially by using complementary color LEDs. The spectral distributions of light, measured at the abaxial side of the tomato leaves while the adaxial side was exposed to the light, showing the emission of chlorophyll fluorescence, as shown in Fig. 9, Fig. 10, and Fig. 11. The chlorophyll fluorescence could be also detected in both the leaves of seedlings and those of mature plants. Even though, the peak of emitted fluorescence could not be detected by this measuring method using other kinds of light sources, e.g., fluorescent lamp (Fig. 19) and solar radiation (Fig. 20) which have higher PF in FR region compared with the LEDs.

3.3.2 Fluorescence yield related to the transmittance

From the results of transmittance and the fluorescence yield, as shown in Fig. 16, Fig. 17 and Fig. 18, the light quality which has the larger transmittance resulted in the smaller fluorescence yield. In other words, the amount of photon absorbed by the leaves has closed relation to the emission of the chlorophyll fluorescence.

The fluorescence emission was changed with the time, leaf age, position on the leaves, air components, etc. (e.g., Briantais et al, 1986), therefore, the precise measurement for the amount of fluorescence emitted from the leaves in relation to those environment factors is still needed.

From the results in Chapter 1, the FR light played roles on stem elongation of seedlings grown under fluorescent light without the increment of dry weight. It might be concluded that not only the FR photon from the light sources but the increased FR ratio from the near-by leaves can also cause the elongation of the seedlings inside the canopy. Moreover, this might be the close relationship between the sun leaves and shading leaves, excluding their responses to the light intensity and light quality.

There was a report that the leaf might recognize the shading conditions by detection of FR ratio. Thus, the increasing in FR ratios of light after transmitted through the tomato leaves could be the useful information for the explanation of elongated seedlings or plants inside the high planting density canopies.

3.4 Conclusions

The decreased PF ratios of B:FR, G:FR, and R:FR are the results from not only the higher transmittance of FR compared with that of the B, G, or R, but also from the emitted FR, which can be detected by using the complementary color LEDs.

The quality of incident light influenced the fluorescence yield emitted from the tomato leaves. Under the same light intensity, green light and the light which has higher green ratio induced the least fluorescence yield compared with the blue and red light, respectively. The differences in absorptance of the leaves against the green, blue, and red light might play role in the fluorescence yield. Even though it is notoriously difficult to measure the absolute absorptance of the leaves, while attempts in measurements of the absorptance were done continuously.

In the past, research has been conducted on focusing the light intensity and its effects on chlorophyll fluorescence. However, in the present study, the effect of light quality at low light intensity on emission of chlorophyll fluorescence was shown.

General Conclusions

In the present study, it can be concluded that:

- (1) Green light was as effective as red light on growth of tomato plug seedlings in term of dry weight.
- (2) Green light is more evenly absorbed in the vertical orientation compared with red and blue lights, therefore, application of green light can increase the probability of light interception inside the canopy.
- (3) The photomorphogenesis and the light interception inside the canopy greatly influenced the growth of plug seedlings.
- (4) The transmission of tomato seedling leaves was dependent upon the quality of light. Transmittance of green light is the higher than those of blue and red light, respectively. Also the light with higher percent green photon flux can effectively penetrate the more leaf layers.
- (5) The emitting far-red (FR) light could be detected at the abaxial side of the tomato leaves when the adaxial side was exposed to the light. The yield of emitting FR light was increasing with the decreasing transmittance.
- (6) The photomorphogenesis and photosynthesis play important roles in the growth of plants with their close relationship. The photomorphogenesis, like leaf expansion and stem elongation, might due to the enhancement of photosynthesis. Also, the enhancement of photosynthesis by increasing the photon flux incident on the leaves resulted in the reduction of elongation, such as increasing of green light PF, side-ward lighting system.

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Tables

Table 1 Photon flux (PF; $\mu\text{mol m}^{-2} \text{s}^{-1}$)* composition in each treatment

Treatment	Wavelength region (nm)				
	400-500	500-600	600-700	400-700	700-800
	Blue PF	Green PF	Red PF	PPF**	Far-red PF
Fluorescent light	55	101	74	230	8.0
G24%	55	55	120	230	0.3
G12%	55	29	146	230	0.4
G1%	55	3	172	230	0.5

*, ** Photon flux or photosynthetic photon flux (PPF; $\mu\text{mol m}^{-2} \text{s}^{-1}$) at the top of canopy level.

Table 2 Relative photon flux (% of PPF) and red/far-red ratio in each treatment

Treatment	Blue*	Green*	Red*	Far-red*	R/FR
Fluorescent light	24.0	44.0	32.0	3.48	10.81
G24%	24.0	24.0	52.0	0.13	0.25
G12%	24.0	12.5	63.5	0.17	0.27
G1%	24.0	1.0	75.0	0.22	0.29

* The relative photon fluxes were basis on PPF.

Table 3 Effects of percent green photon flux on fresh weight, dry weight, stem length and shoot dry weight per stem length ratio of tomato plug seedlings on day 15 after sowing

Treatment	Total FW (g)	Total DW (mg)	Stem L (mm)	Shoot DW/stem L (g m ⁻¹)
Flu. 1 ^x	0.85±0.05 ^z	62.5±5.2	93±3.5	6.2±0.52
G24%	0.83±0.08	65.9±6.8	82±3.4	7.3±0.69
t-test	NS ^y	NS	**	**
Flu. 2 ^w	1.17±0.04	68.8±4.9	117±1.4	5.5±0.40
G12%	1.27±0.05	72.3±6.5	108±4.3	6.4±0.67
t-test	**	NS	**	*
Flu. 3 ^v	0.85±0.05	60.2±6.2	88±1.9	6.2±0.69
G1%	1.00±0.05	69.4±5.3	87±2.3	7.3±0.58
t-test	**	*	NS	*

^z: Mean±standard deviation (n=8)

^y: NS, *, **, Non-significant or significant difference at $P = 0.05, 0.01$ respectively

^x, ^w, ^v: The Flu. treatments conducted during the same time with G24%, G12%, or G1%, respectively

Table 3 (cont.) Effects of percent green photon flux on epi-/hypocotyl length ratio and root of tomato plug seedlings on day 15 after sowing

Treatment	Epi-/hypocotyl L ratio	Root FW (g)	Root DW (mg)	Root L (mm)
Flu. 1 ^x	0.80±0.05 ^z	101±0.01	5.8±0.8	78±7
G24%	0.79±0.08	103±0.02	6.1±1.0	75±6
t-test	NS ^y	NS	NS	NS
Flu. 2 ^w	0.84±0.06	89±0.02	3.8±0.5	64±14
G12%	0.79±0.06	90±0.02	4.4±0.9	58±7
t-test	NS	NS	*	NS
Flu. 3 ^v	0.66±0.03	102±0.01	4.9±0.8	70±14
G1%	0.72±0.07	113±0.02	5.6±0.7	66±6
t-test	NS	*	NS	NS

^z: Mean±standard deviation (n=8)

^y: NS, *, **, Non-significant or significant difference at $P = 0.05, 0.01$ respectively

^x, ^w, ^v: The Flu. treatments conducted during the same time with G24%, G12%, or G1%, respectively

Table 3 (cont.) Effects of percent green photon flux on cotyledons and leaves of tomato plug seedlings on day 15 after sowing

Treatment	Cotyledon DW (mg)	Cotyledon area (cm ²)	Leaf DW (mg)	Leaf area (cm ²)
Flu. 1 ^x	10.3±1.7 ^z	4.3±0.5	28.4±2.3	17.0±1.1
G24%	10.9±1.7	4.2±0.5	32.0±3.2	16.9±0.9
t-test	NS ^y	NS	**	NS
Flu. 2 ^w	10.5±1.0	5.5±0.3	32.9±2.9	23.5±1.4
G12%	10.5±1.3	5.4±0.4	33.1±3.2	23.0±1.4
t-test	NS	NS	NS	NS
Flu. 3 ^v	10.0±1.5	4.6±0.5	27.0±2.3	16.4±0.6
G1%	11.7±1.5	5.3±0.3	31.9±2.4	18.6±0.8
t-test	NS	**	**	**

^z: Mean±standard deviation (n=8)

^y: NS, **, Non-significant or significant difference at $P = 0.01$ respectively

^x, ^w, ^v: The Flu. treatments conducted during the same time with G24%, G12%, or G1%, respectively

Table 4 Relative growth of tomato plug seedlings on day 15 after sowing

Treatment	Total FW	Total DW	Stem L
G24%	97.9±7.1 ^z	105.7± 9.7	88.5±5.8
G12%	109.2±2.1	105.3± 9.3	92.3±3.7
G1%	117.0±9.9	116.4±15.2	98.9±3.1
LSD _{P = 0.05}	7.4	13.0	12.9

^z: Mean±standard deviation (n=8)

Table 4 (cont.) Relative growth of tomato plug seedlings on day 15 after sowing

Treatment	Cotyledon area	Leaf area
G24%	100.3±12.5 ^z	100.5±5.5
G12%	99.0± 9.5	98.5±9.7
G1%	116.6±15.4	113.4±7.6
LSD _{P = 0.05}	14.1	13.0

^z: Mean±standard deviation (n=8)

Table 5 Specific growth of tomato plug seedlings on day 15 after sowing

Treatment	Total FW (/100)	Total DW (/100)	Stem L (/100)	Leaf DW (/100)
G24%	-2.1 ± 6.7^z	5.7 ± 9.7	-12.5 ± 6.2	4.5 ± 3.2
G12%	9.2 ± 2.0	5.3 ± 9.3	-6.6 ± 3.1	0.4 ± 3.4
G1%	16.9 ± 9.2	16.4 ± 15.2	-1.3 ± 3.6	7.0 ± 4.5
LSD _{P = 0.05}	7.4	13.0	12.9	5.0

^z: Mean $\pm$ standard deviation (n=8)

Table 5 (cont.) Specific growth of tomato plug seedlings on day 15 after sowing

Treatment	Total FW (/100)	Total DW (/100)	Stem L (/100)	Leaf DW (/100)
G24%	-2.1 ± 6.7^z	5.7 ± 9.7	-12.5 ± 6.2	4.5 ± 3.2
G12%	9.2 ± 2.0	5.3 ± 9.3	-6.6 ± 3.1	0.4 ± 3.4
G1%	16.9 ± 9.2	16.4 ± 15.2	-1.3 ± 3.6	7.0 ± 4.5
LSD _{P = 0.05}	7.4	13.0	12.9	5.0

^z: Mean $\pm$ standard deviation (n=8)

Table 6 Transmittance (%) of tomato leaves in each wavelength region

Wavelength region	Seedlings			Mature plants	
	First ^z	Second ^y	Double ^x	First	Second
400-500 nm	2.4	53	1.3	0.5	-0- ^w
500-600 nm	10.0	42	4.2	6.0	-0-
600-700 nm	4.0	49	2.1	2.0	-0-

^{z,y,x} First or second single leaves from the light source or double leaves

^w Too small data considered by the sensitivity of the photo-sensor

Table 7 PPF transmittance (%) under tomato leaves

Light quality	Seedlings			Mature plants	
	First ^z	Second ^y	Double ^x	First	Second
Blue	2.4	53	1.3	0.5	-0- ^w
Green	10.0	42	4.2	6.0	-0-
Red	4.0	49	2.1	2.0	-0-
G24%	5.4	44	2.4	2.6	-0-
G12%	4.8	44	2.1	2.3	-0-
G1%	3.7	46	1.7	2.0	-0-

^{z,y,x} First or second single leaves from the light source or double leaves

^w Too small data considered by the sensitivity of the photo-sensor

Figures

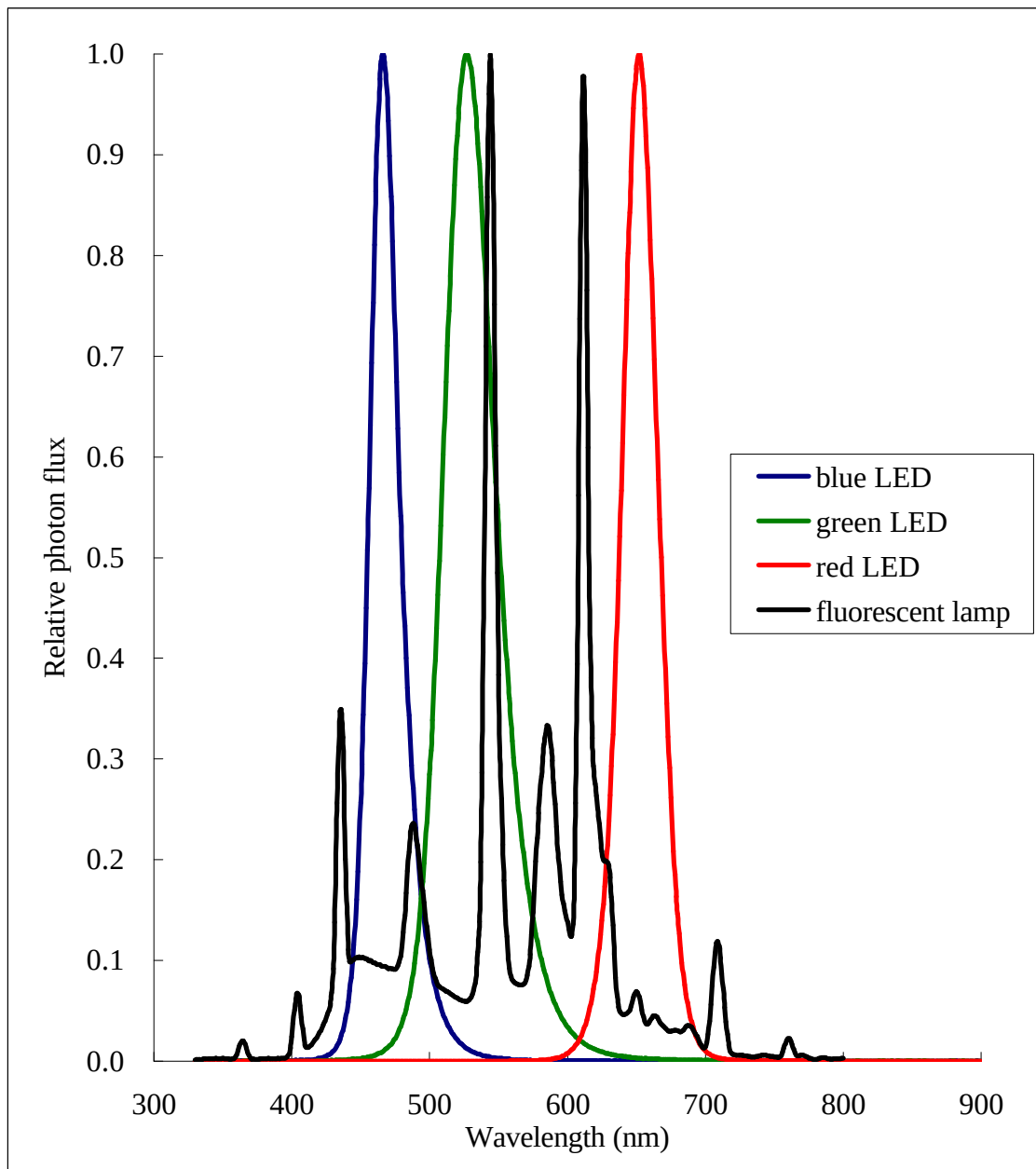


Fig. 1 Spectral distributions in relative photon flux of blue LED (peak at 475 nm), green LED (peak at 535 nm), red LED (peak at 650 nm) and fluorescent lamp used in the present study. Portable spectroradiometer LI-1800, LI-COR Inc., USA) was used for the measurements.

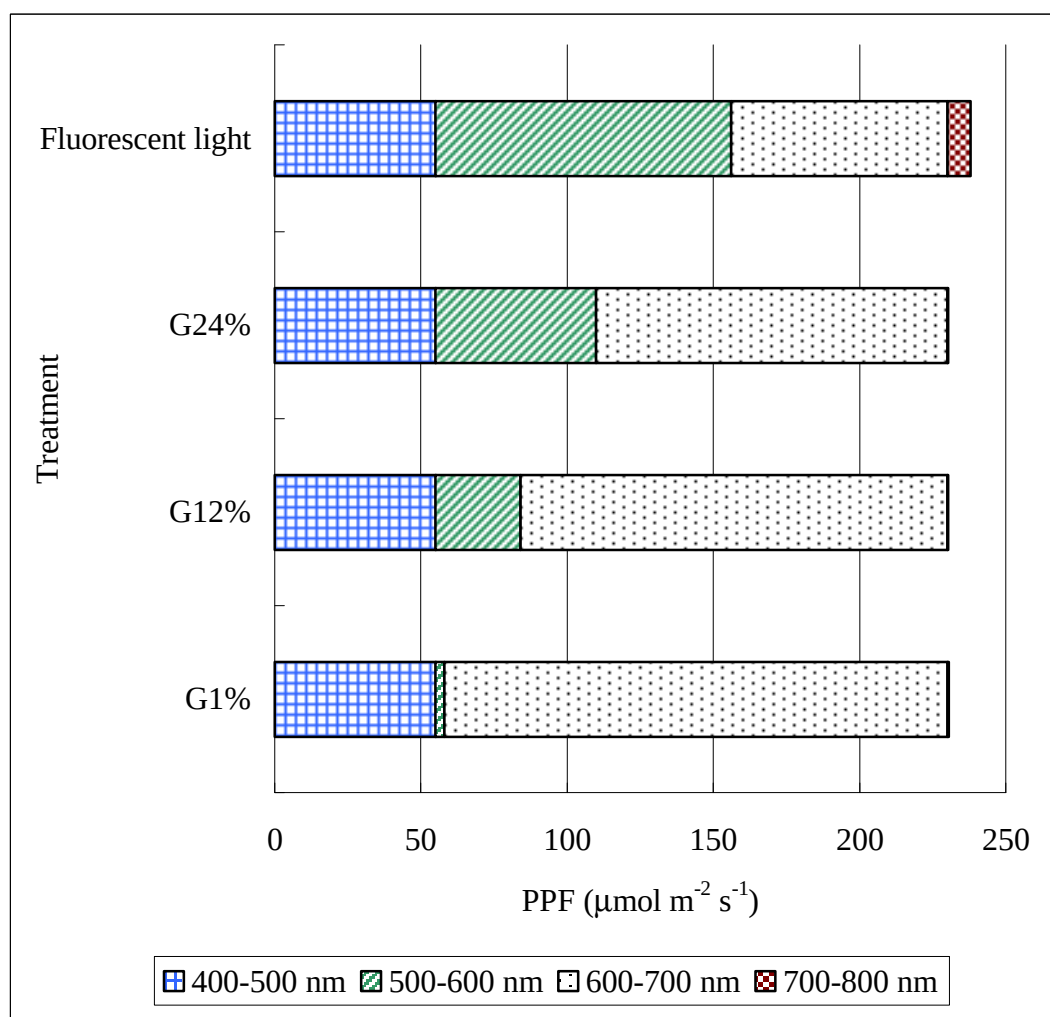


Fig. 2 Photon flux composition of light in each treatment. For treatment codes, see Table 1

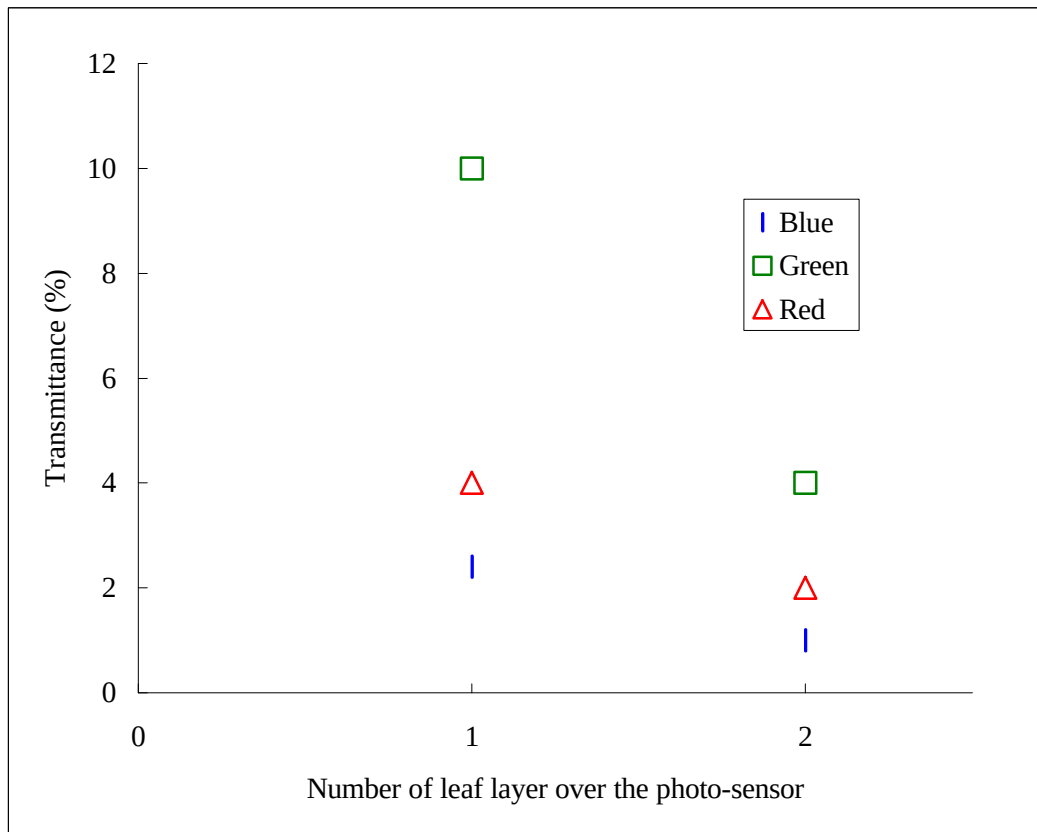


Fig. 3 Transmittance (%) of the complementary light upon the leaves of tomato seedlings. The light sources were green LED (peak at 535 nm), blue LED (peak at 475 nm), and red LED (peak at 650 nm). Blue, green and red regions were the wavelength regions of 400-500 nm, 500-600 nm, 600-700 nm, respectively.

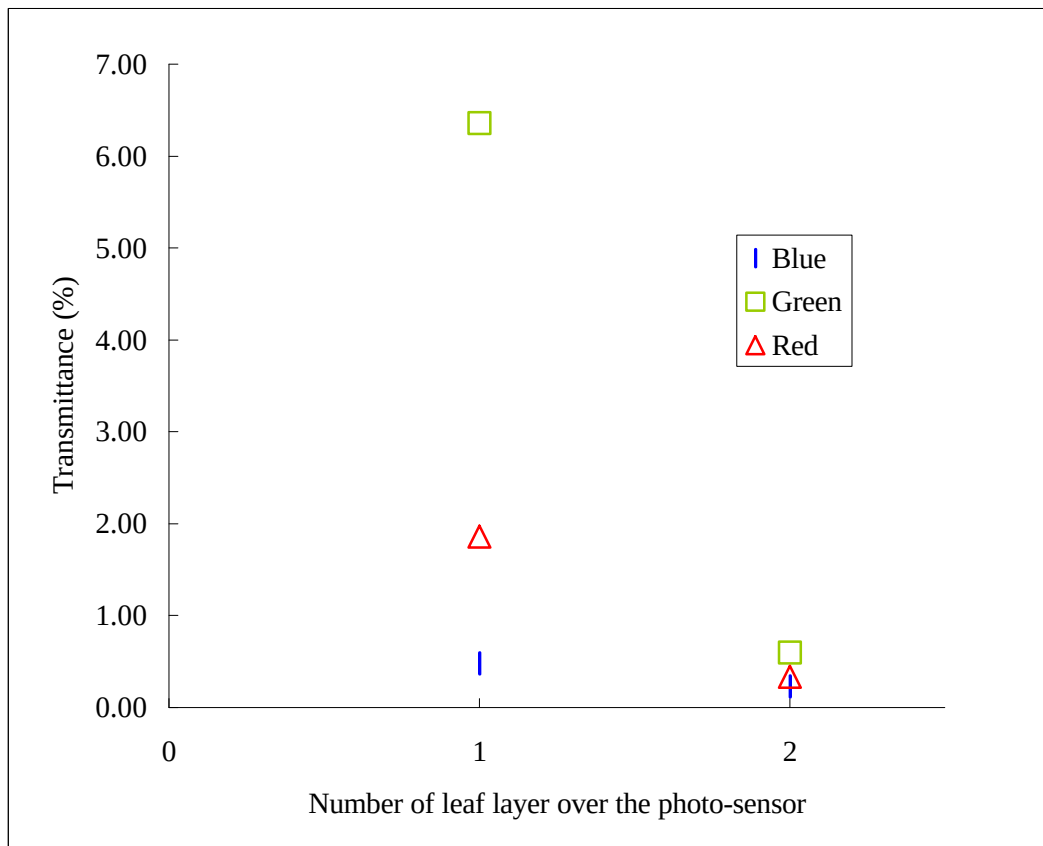


Fig. 4 Transmittance (%) of the complementary light upon the leaves of mature tomato plants. The light sources and wavelength regions of light are mentioned in Fig. 3.

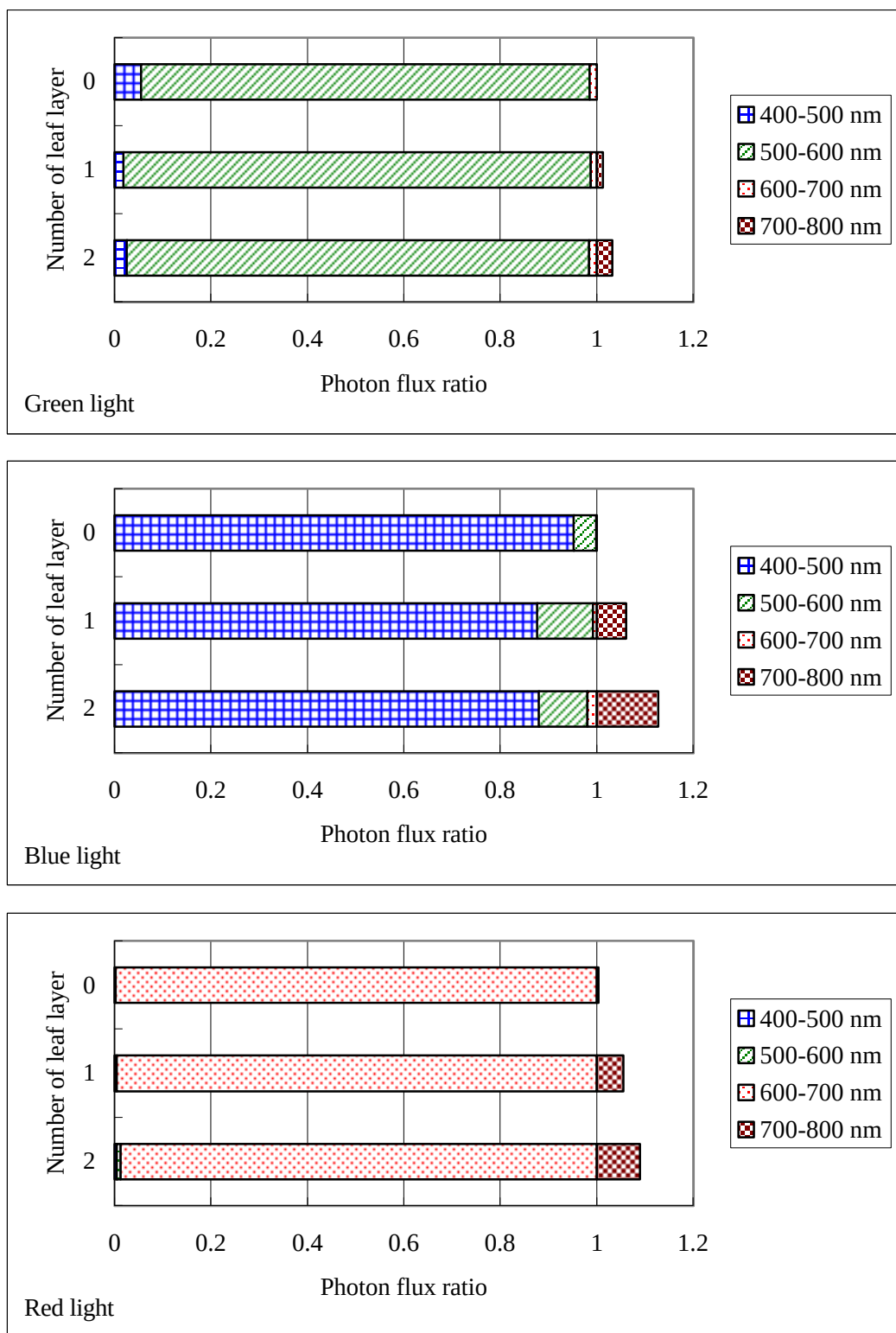


Fig. 5 Changes in photon flux ratio of the green, blue and red light transmitted through the single leaves of tomato seedlings. Number of leaf layer over the photo-sensor varied for 0, 1 or 2 layers.

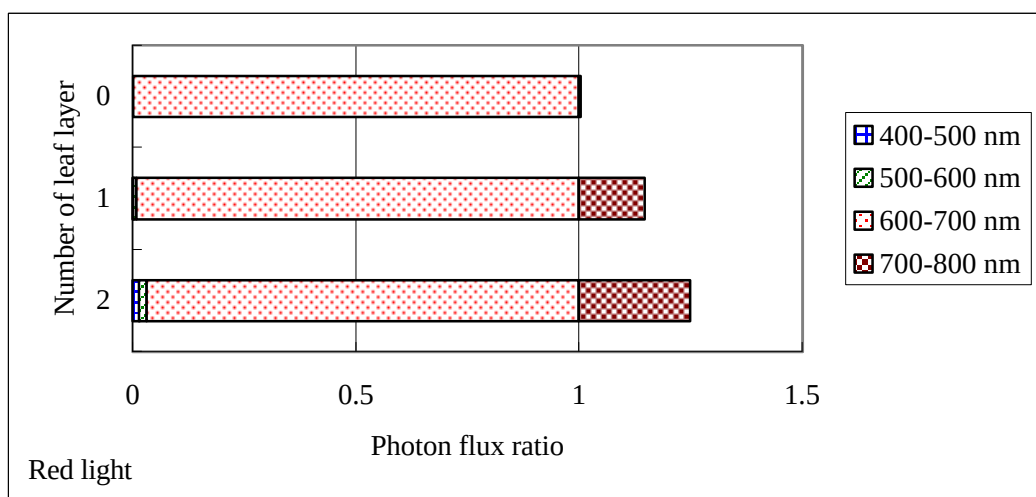
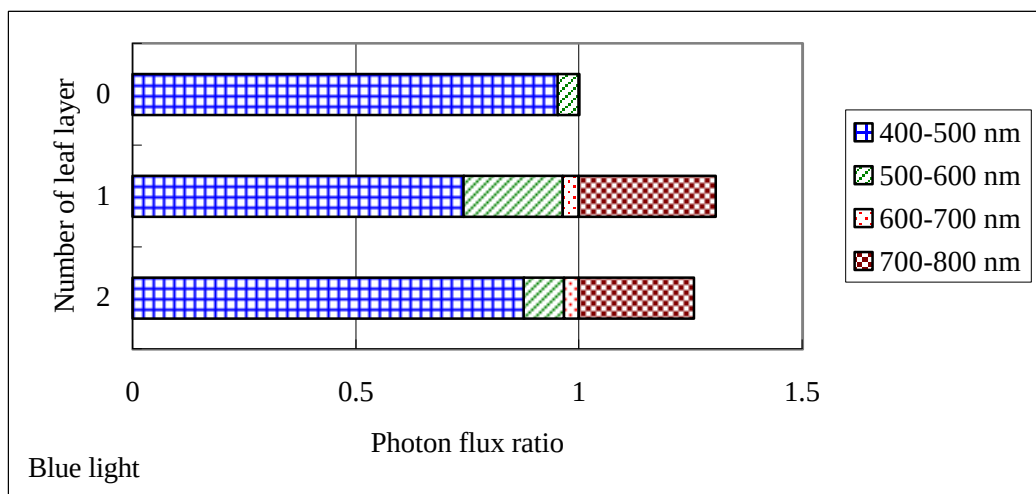
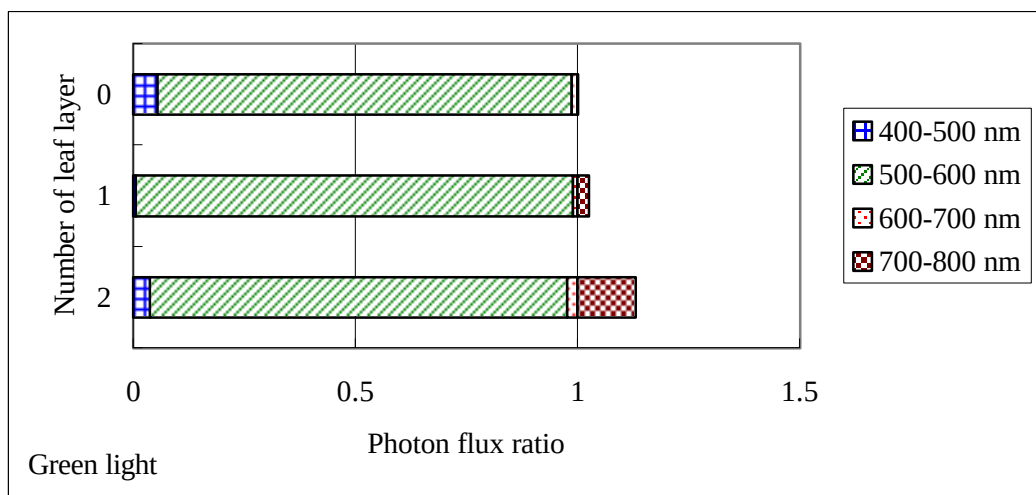


Fig. 6 Changes in photon flux ratio of the green, blue and red light transmitted through the single leaves of tomato mature plants. Number of leaf layer over the photo-sensor varied for 0, 1 or 2 layers.

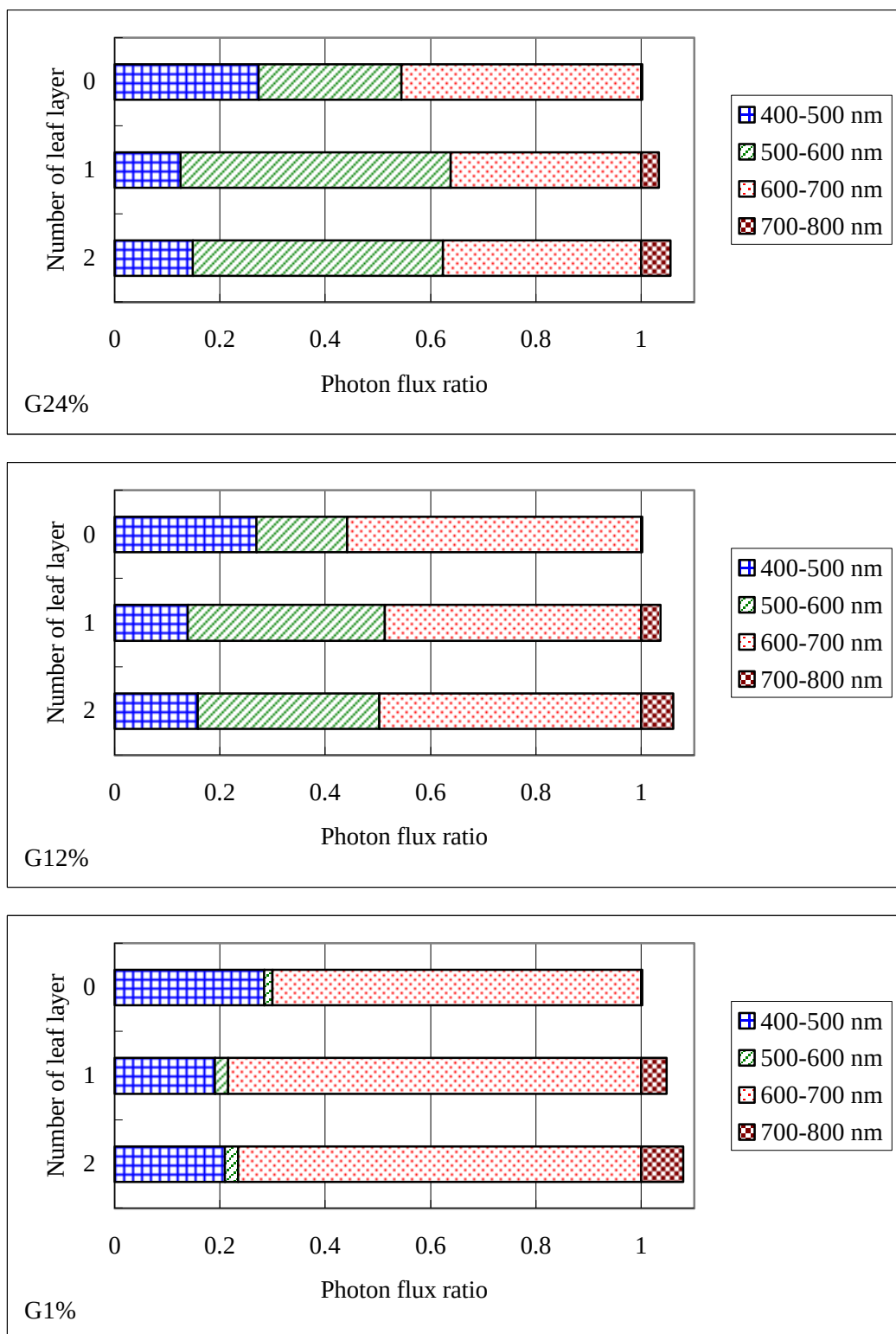


Fig. 7 Changes in photon flux ratio of the G24%, G12% and G1% light transmitted through the single leaves of tomato seedlings. Number of leaf layer over the photo-sensor varied for 0, 1 or 2 layers. For the treatment codes, see Table 1.

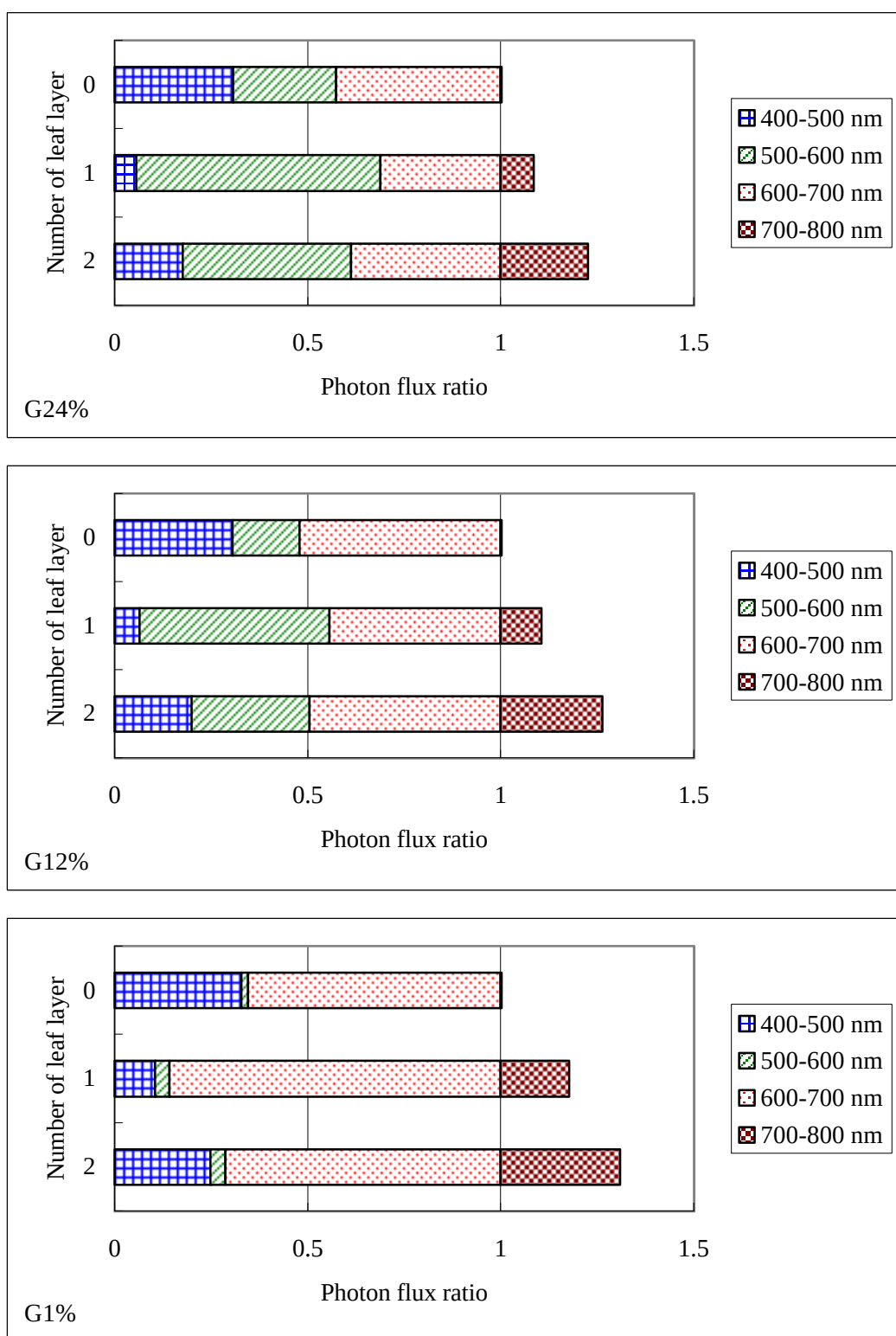


Fig. 8 Changes in photon flux ratio of the G24%, G12% and G1% light transmitted through the single leaves of tomato mature plants. Number of leaf layer over the photo-sensor varied for 0, 1 or 2 layers. For the treatment codes, see Table 1.

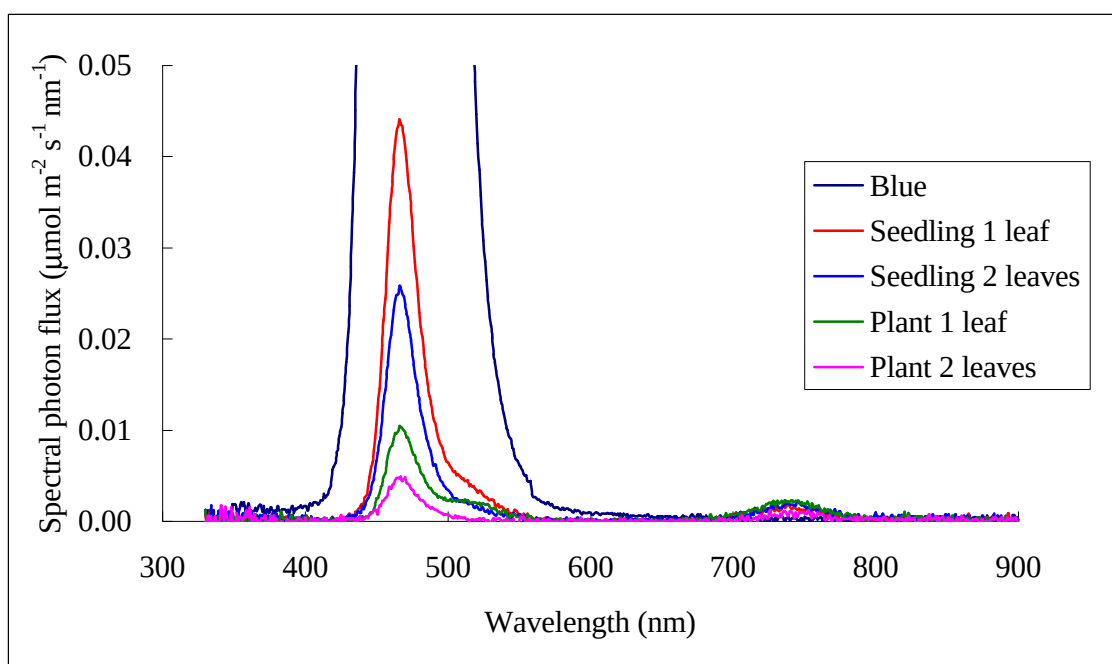
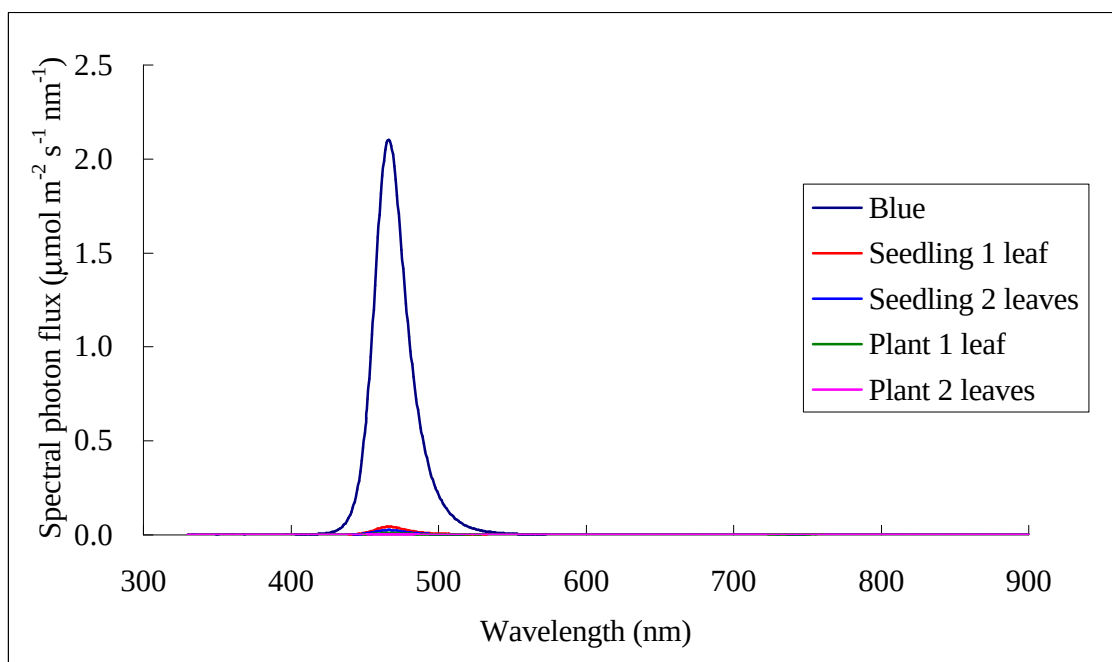


Fig. 9 Spectral distributions of blue light transmitted through (1) single leaves and (2) double leaves of tomato seedlings and tomato mature plants. Light source was blue LED (peak at 475 nm).

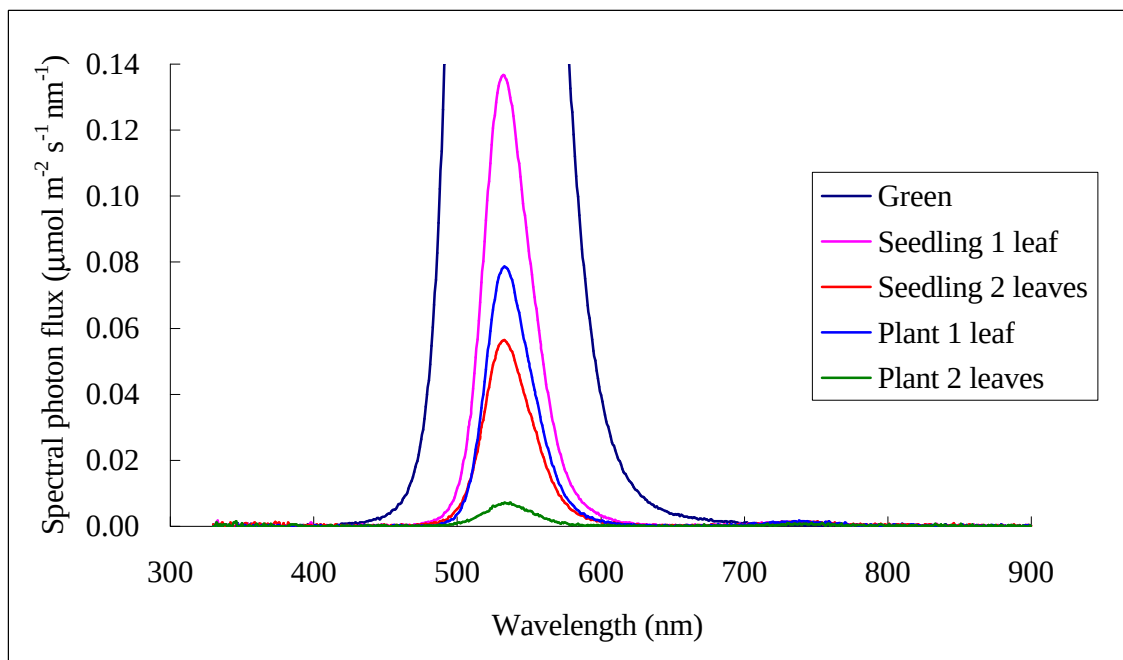
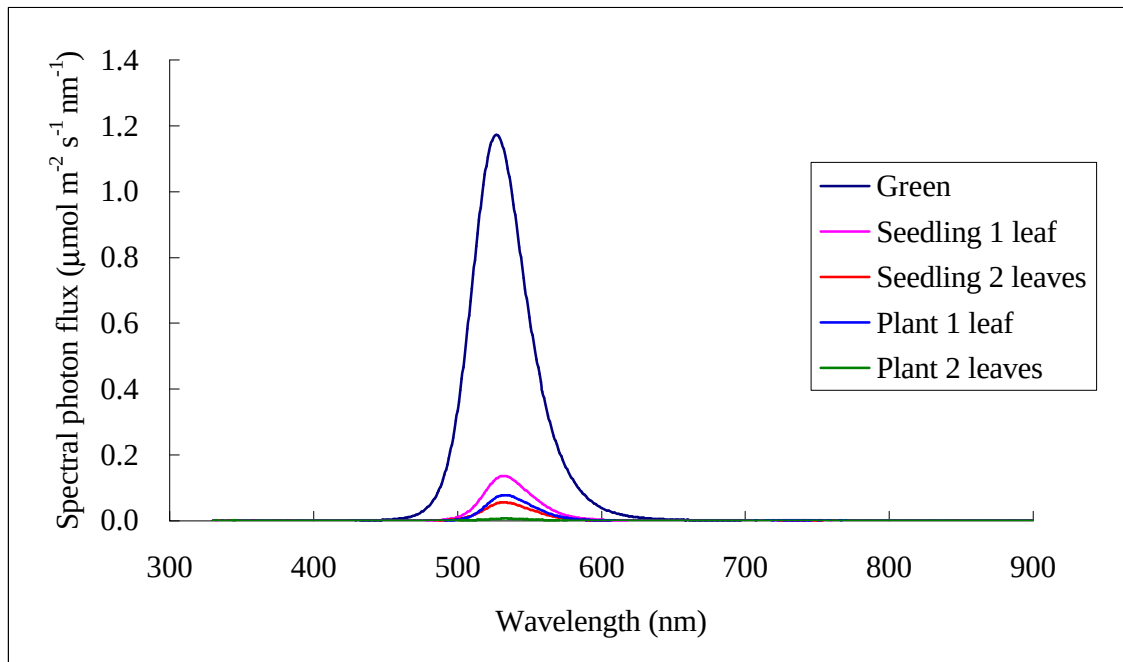


Fig. 10 Spectral distributions of green light transmitted through (1) single leaves and (2) double leaves of tomato seedlings and tomato mature plants. Light source was green LED (peak at 535 nm).

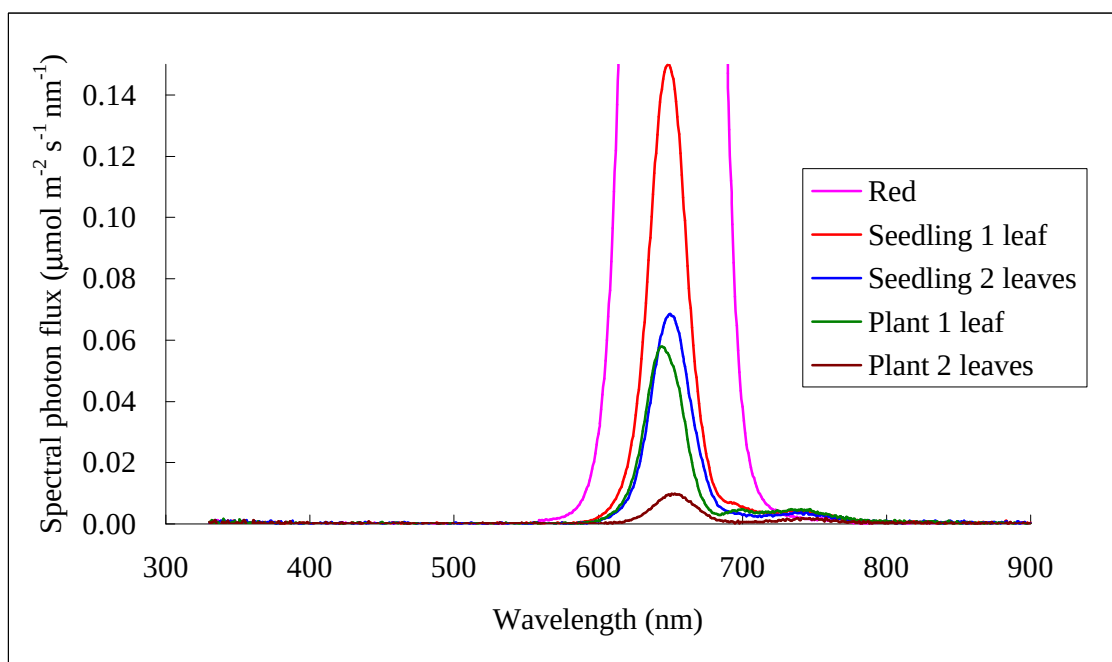
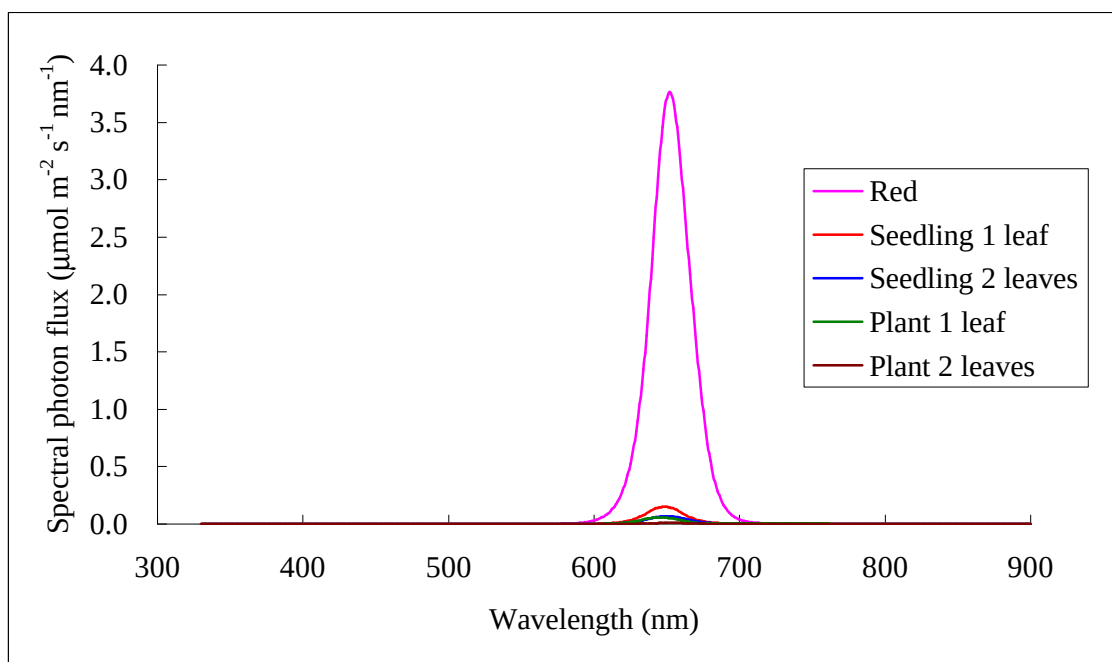


Fig. 11 Spectral distributions of red light transmitted through (1) single leaves and (2) double leaves of tomato seedlings and tomato mature plants. Light source was red LED (peak at 650 nm).

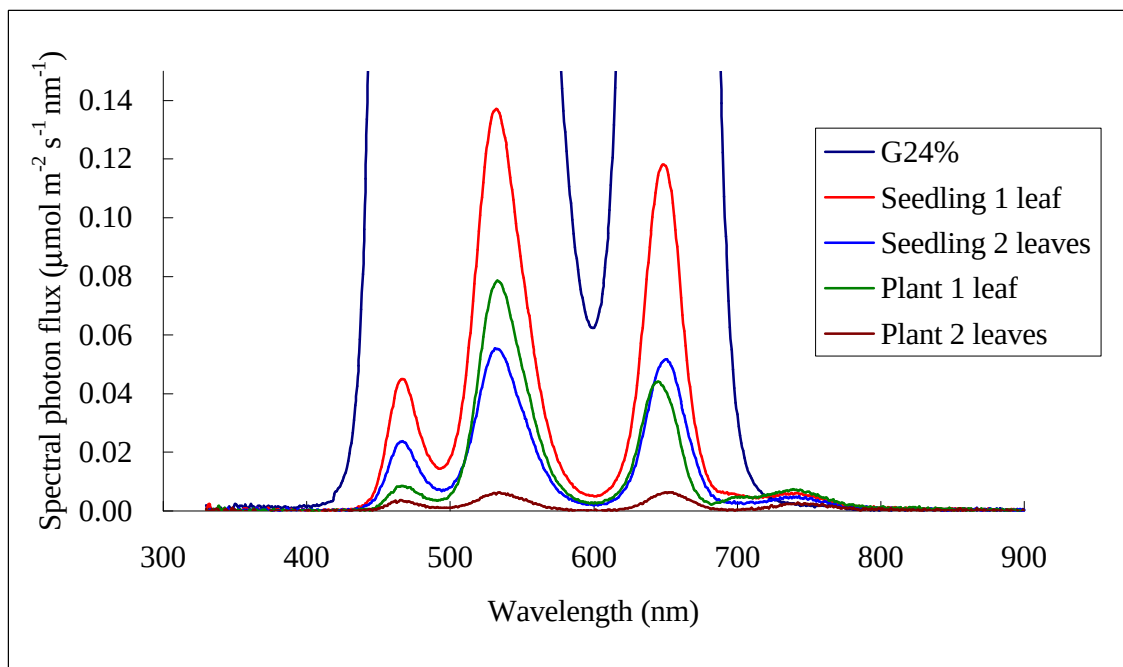
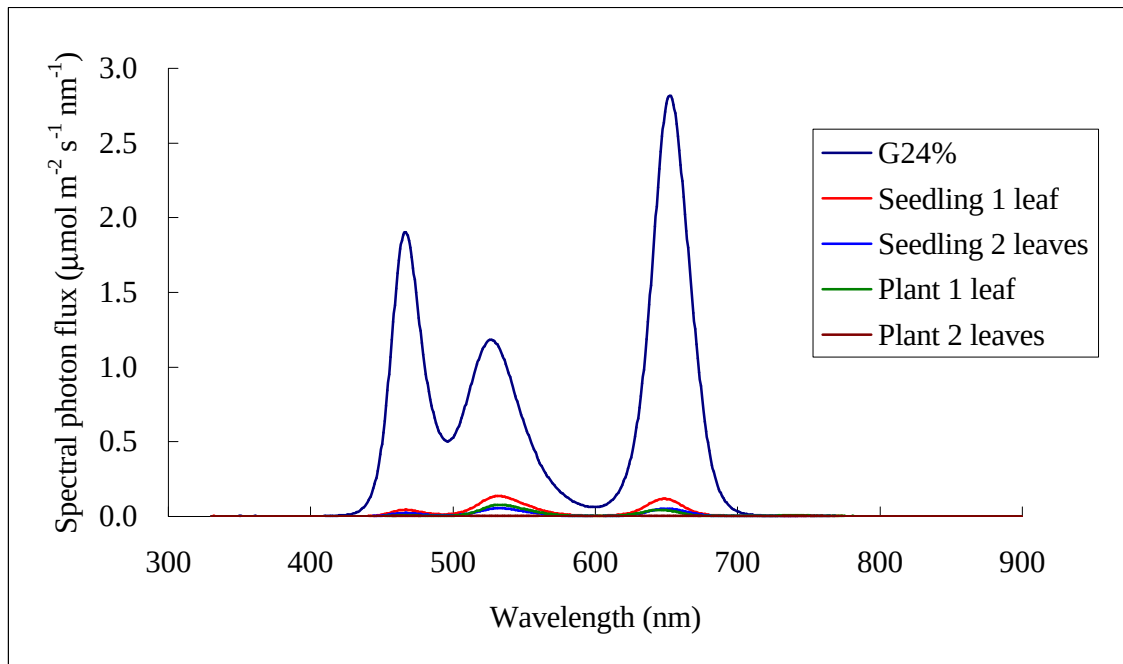


Fig. 12 Spectral distributions of G24% light transmitted through (1) single leaves and (2) double leaves of tomato seedlings and tomato mature plants. Light source was blue LED (peak at 475 nm), green LED (peak at 535 nm), and red LED (peak at 650 nm).

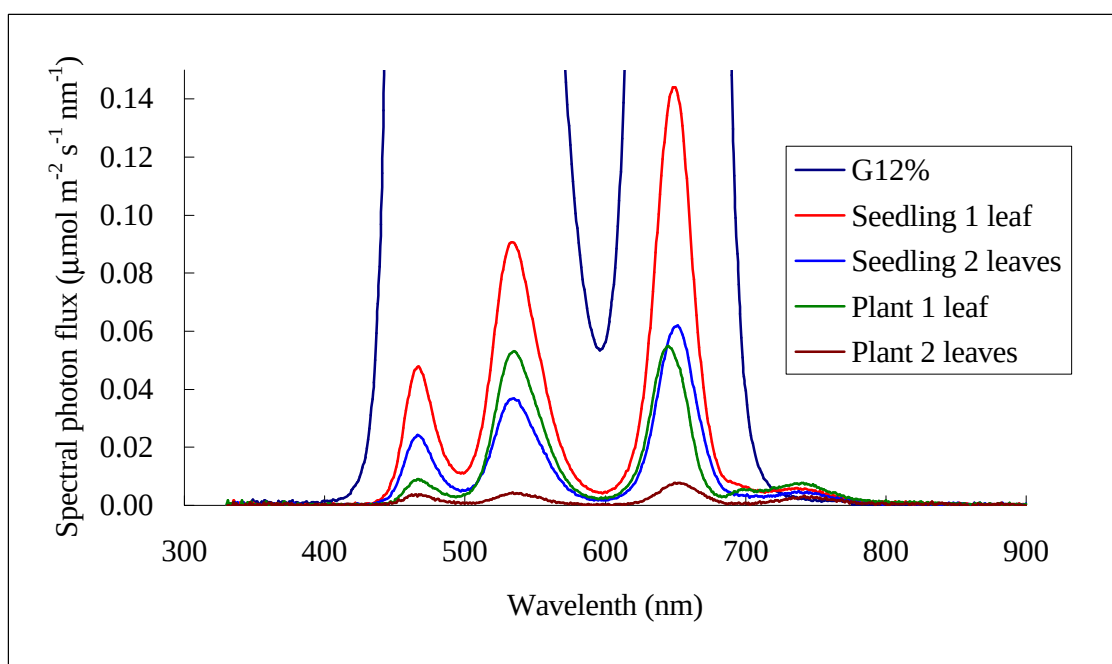
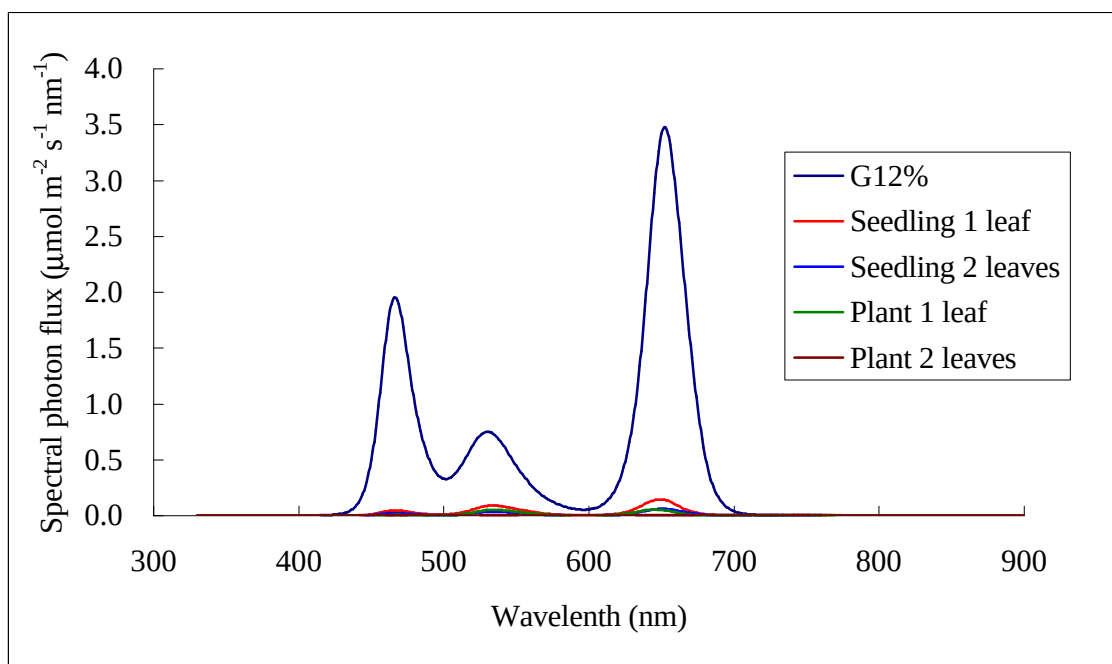


Fig. 13 Spectral distributions of G12% light transmitted through (1) single leaves and (2) double leaves of tomato seedlings and tomato mature plants. Light source was blue LED (peak at 475 nm), green LED (peak at 535 nm), and red LED (peak at 650 nm).

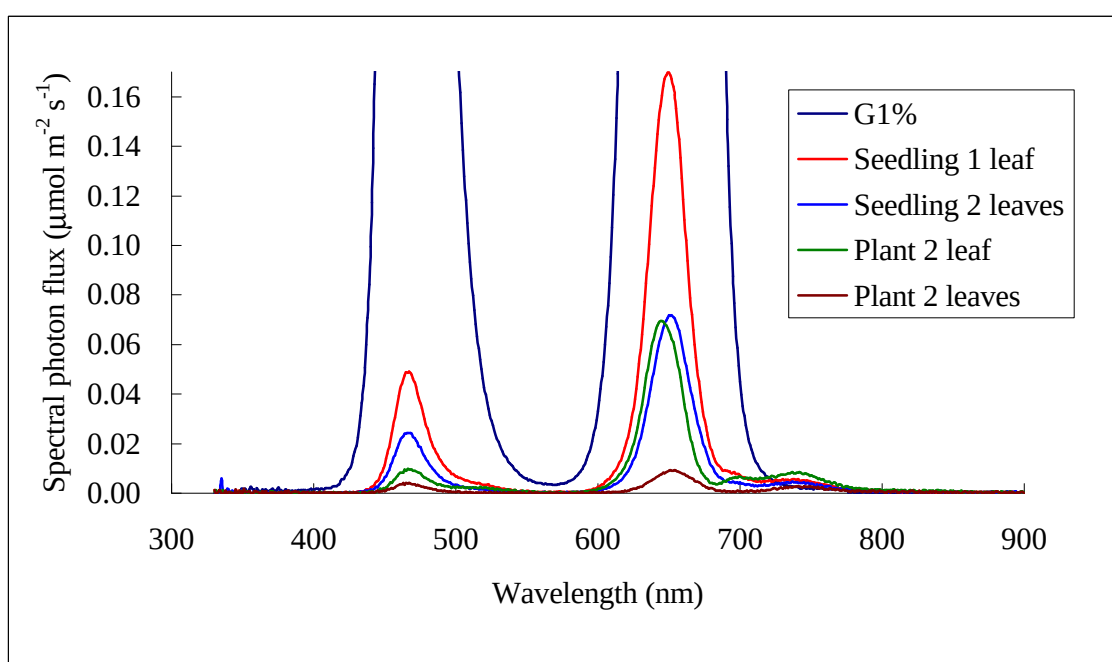
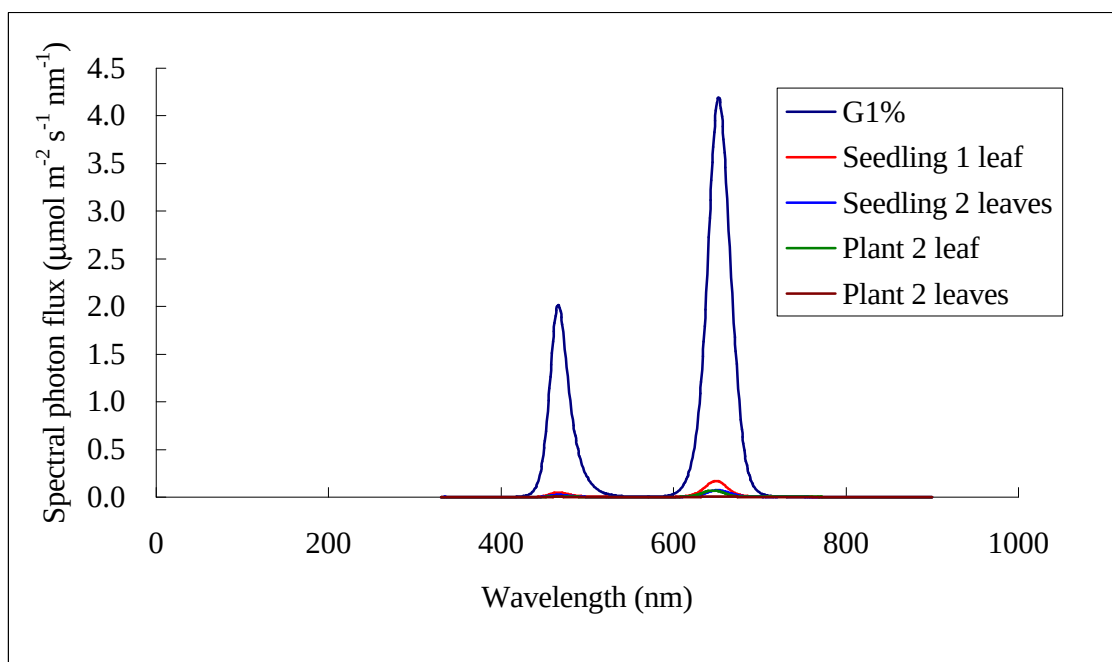


Fig. 14 Spectral distributions of G1% light transmitted through (1) single leaves and (2) double leaves of tomato seedlings and tomato mature plants. Light source was blue LED (peak at 475 nm), green LED (peak at 535 nm), and red LED (peak at 650 nm).

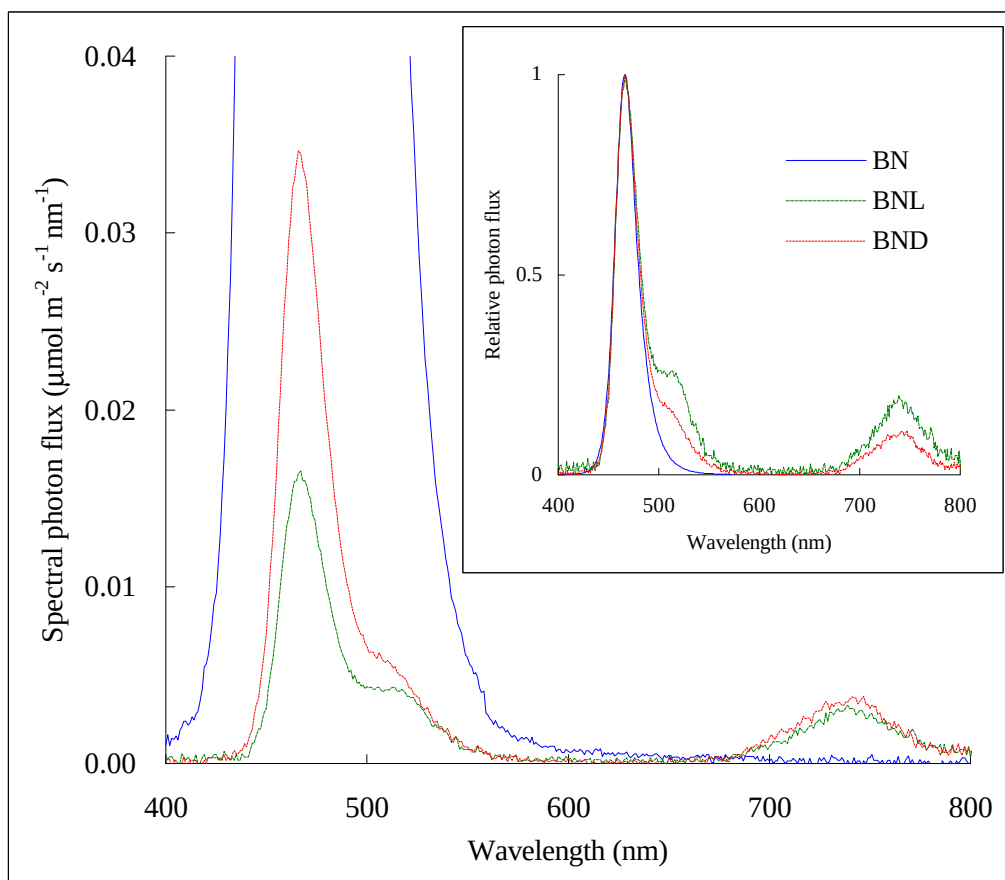
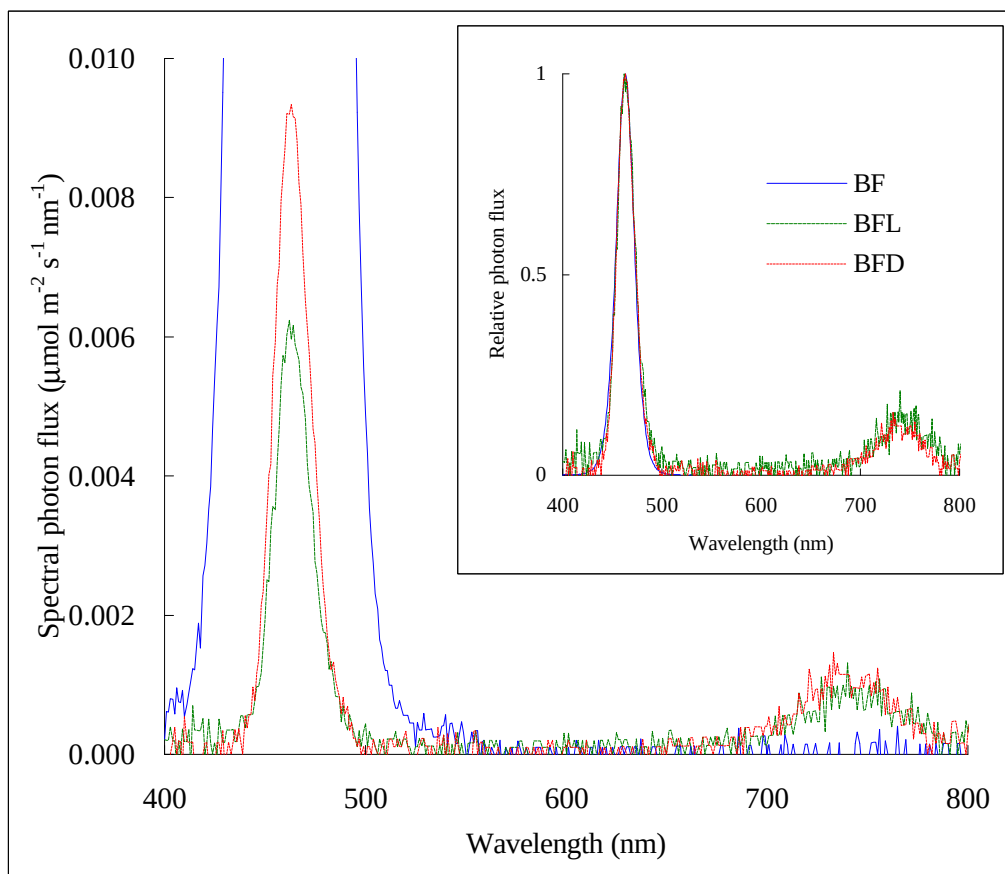


Fig. 15 The shoulder of peak detected in the spectral distribution of blue LED after transmitted through the sweetpotato leaves. Light source used in the upper figures was blue LED without any filters. Light source used in the lower figures was blue LED after filtered by the blue filter (Hoya Co. Ltd., Japan). The smaller figures show spectral distributions in relative photon flux. The bigger figured show spectral distribution in spectral photon flux. BN and BF indicate spectral distributions of incident light without and with blue filters, respectively. L indicates incident leaves and D indicates dehydrated leaves.

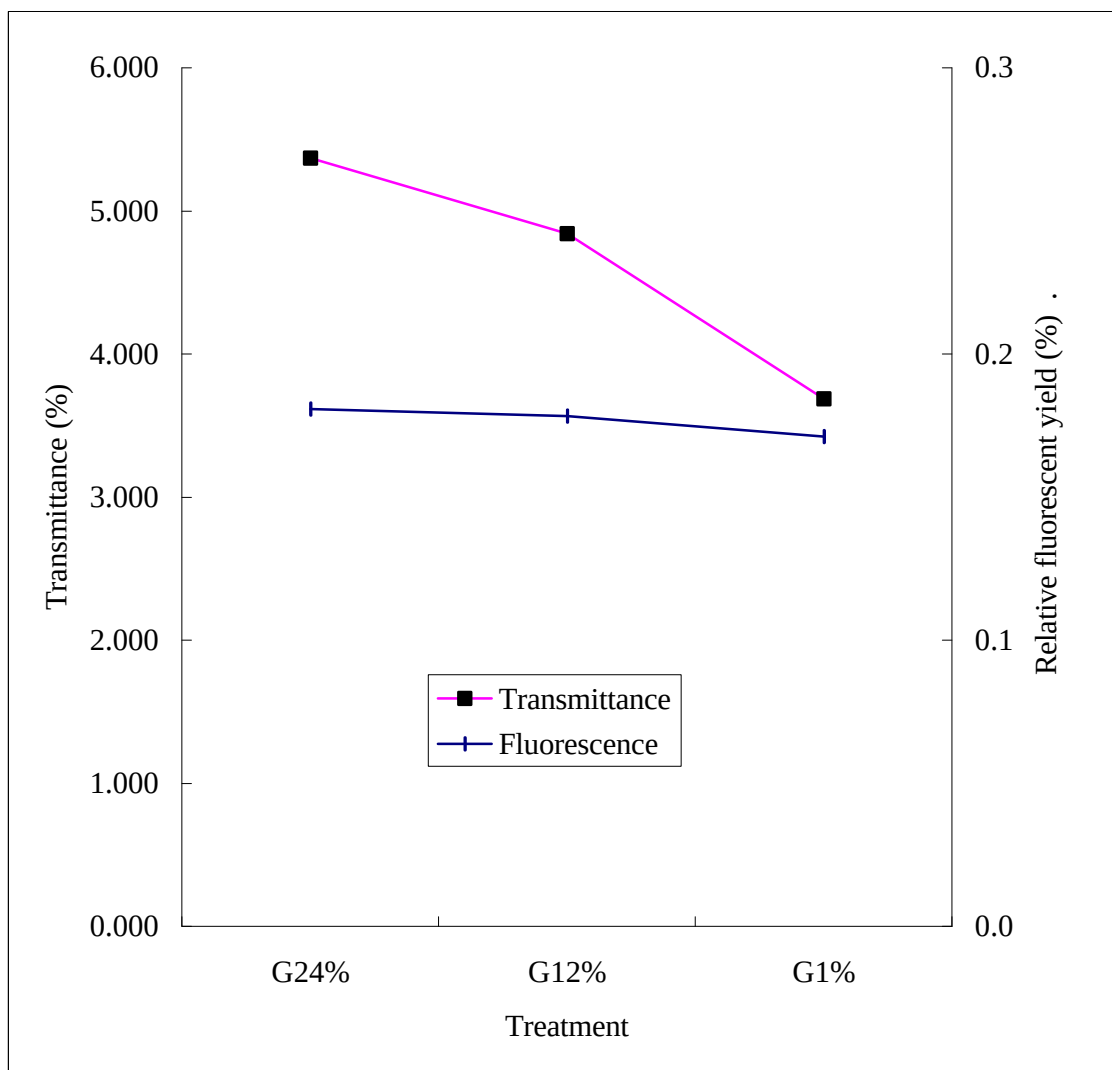


Fig. 16 The relationship between transmittance (%) and relative fluorescent yield (%) of the single leaves of tomato seedlings. Light sources was blue LED (peak at 475 nm), green LED (peak at 535 nm), and red LED (peak at 650 nm). For the treatment codes, see Table 1.

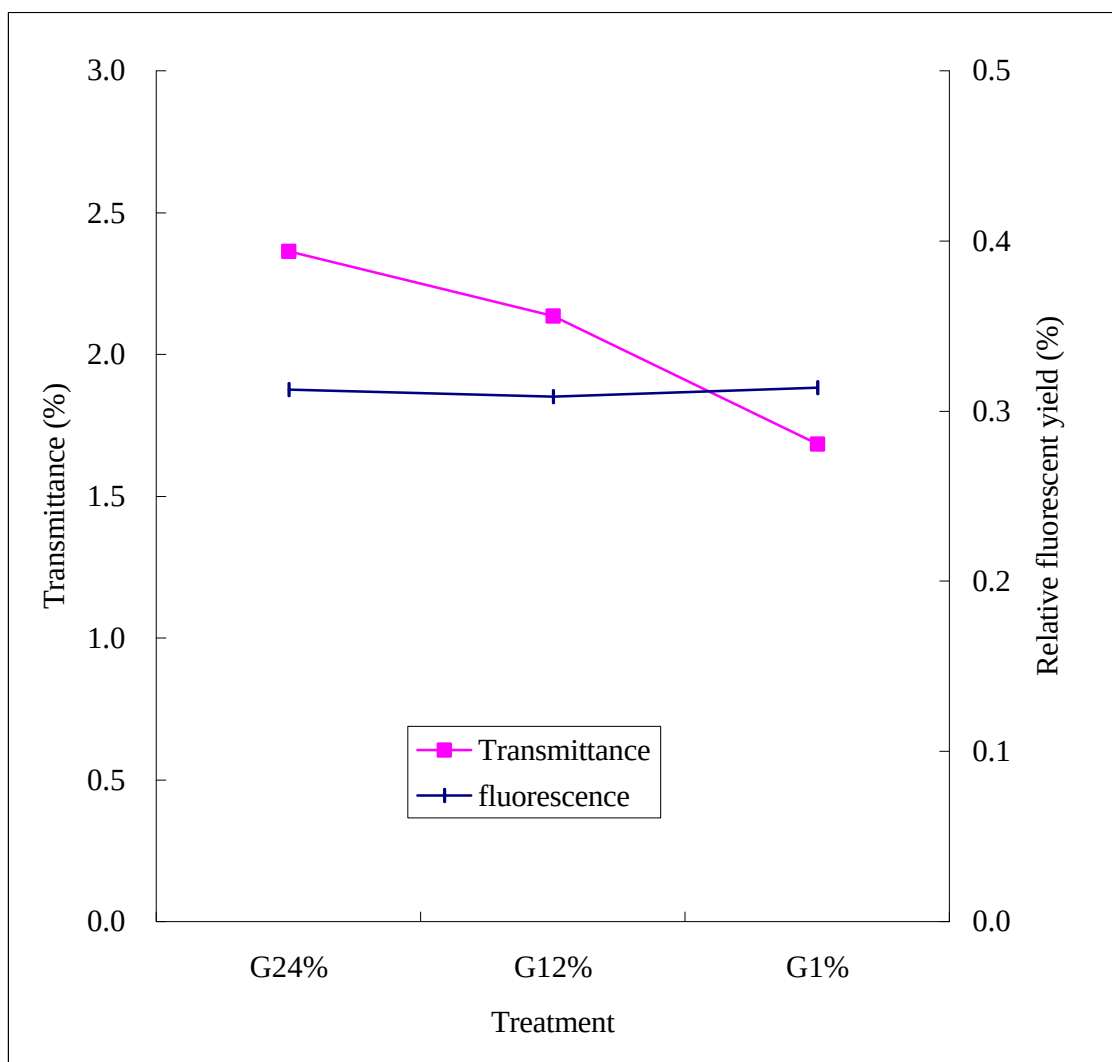


Fig. 17 The relationship between transmittance (%) and relative fluorescent yield (%) of double leaves of tomato seedlings. Light sources was blue LED (peak at 475 nm), green LED (peak at 535 nm), and red LED (peak at 650 nm). For the treatment codes, see Table 1.

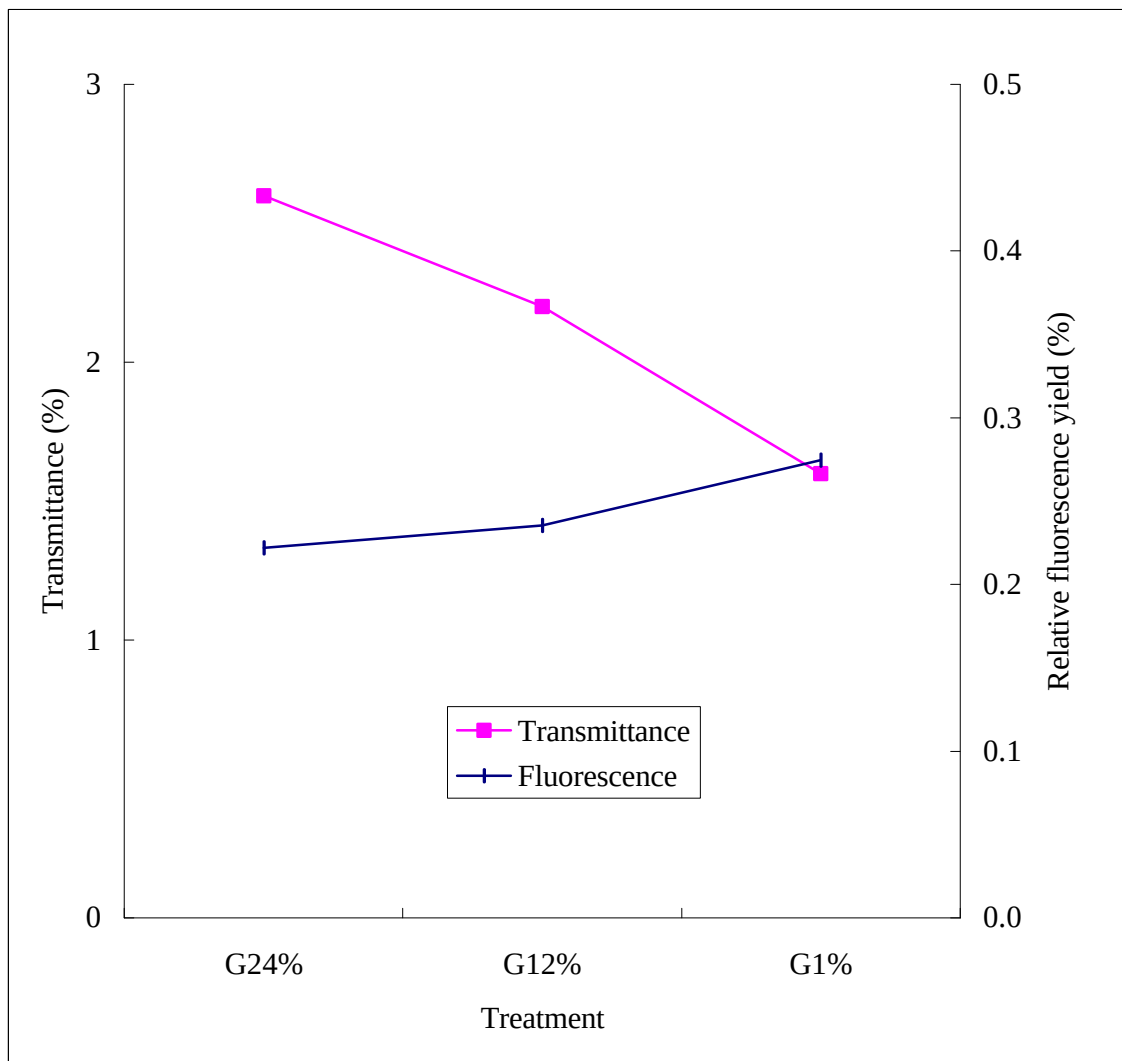


Fig. 18 The relationship between transmittance (%) and relative fluorescent yield (%) of double leaves of tomato mature plants. Light sources was blue LED (peak at 475 nm), green LED (peak at 535 nm), and red LED (peak at 650 nm). For the treatment codes, see Table 1.

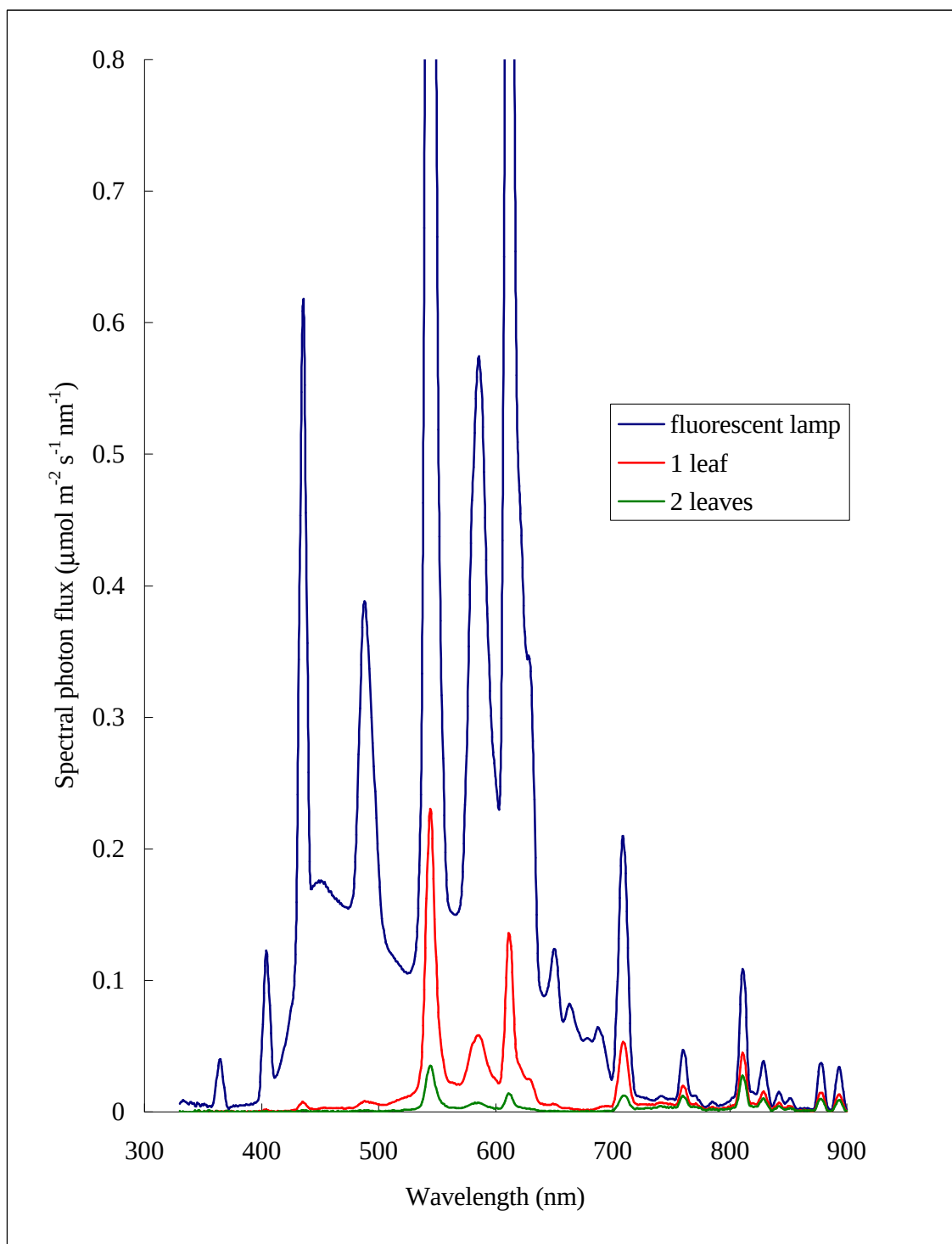


Fig. 19 Spectral distributions of the fluorescent lamp and fluorescent light transmitted through the 1 (single) or 2 (double) leaves of tomato seedlings.

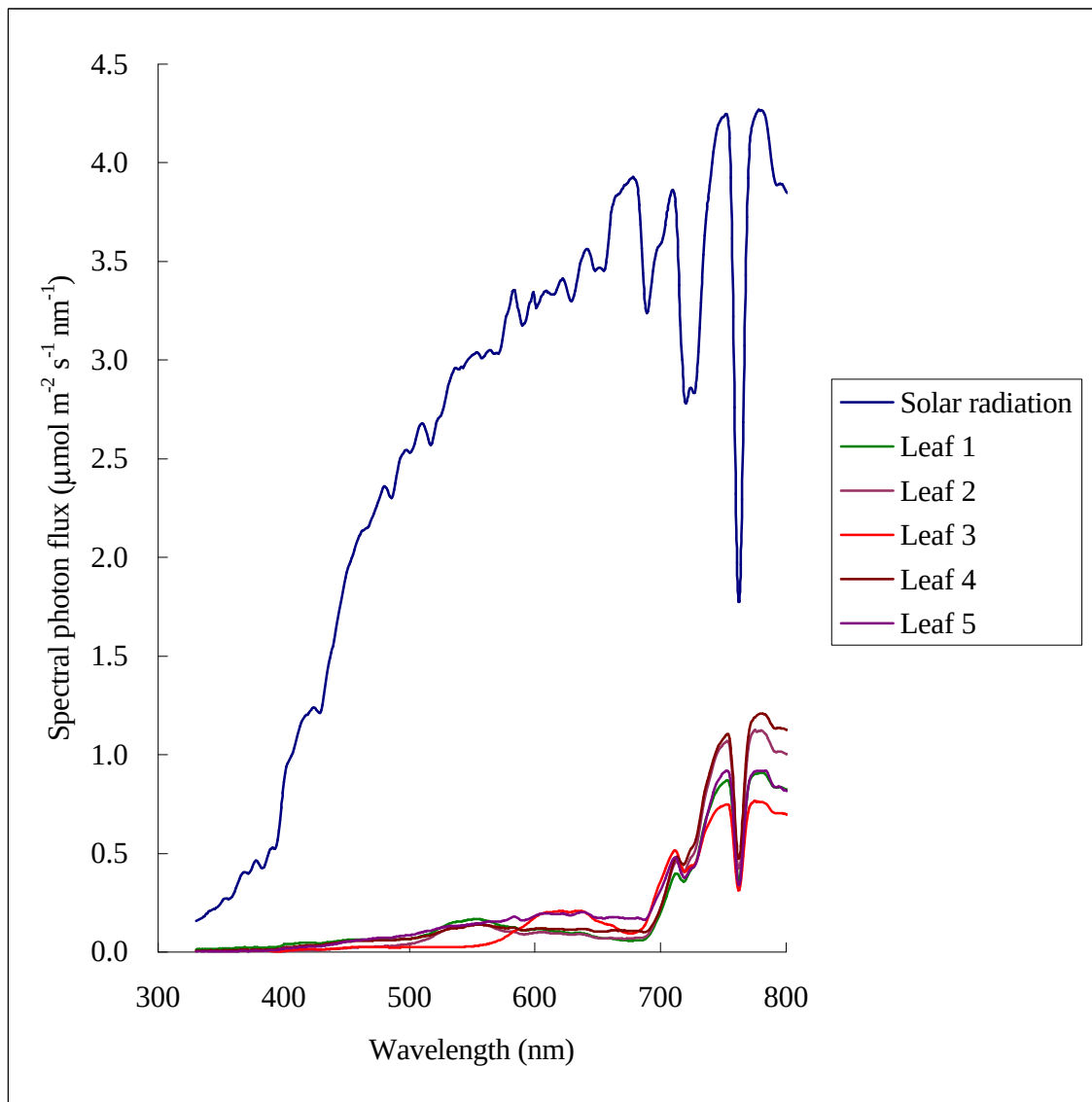


Fig. 20 Spectral distributions of clear-sky solar radiation and the solar radiation after transmitted through single leaves.

Appendixes

Appendix 1 Schematic diagram of experimental setup for the LED box

Appendix 2 Seedlings grown under G24% on day 15 after sowing compared with the seedlings grown under fluorescent light on day 15 after sowing. Environmental condition for both were the same.

Appendix 3 Seedlings grown under G12% on day 15 after sowing compared with the seedlings grown under fluorescent light on day 15 after sowing. Environmental conditions for both were the same.

Appendix 4 Seedlings grown under G1% on day 15 after sowing compared with the seedlings grown under fluorescent light on day 15 after sowing. Environmental conditions for both were the same.

Appendix 5 Portable spectroradiometer (LI-1800, LICOR Inc., USA) used for measurement of spectral distributions.

Appendix 6 Solid state relay used for converting digital signal from data logger (appendix 7) to on/off signal controlling heaters and fans

Appendix 7 Data logger (21X, Campbell Co. Ltd., USA)

Appendix 8 Program of data logger used for controlling heaters and fans inside LED box and fluorescent lamp box

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